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THE UNIVERSITY OF ALBERTA

DEVELOPMENT AND REPRODUCTIVE BIOLOGY OF THREE SPECIES OF *SOLASTER* SEA STARS FROM THE NORTHEAST PACIFIC OCEAN.

BY

SALLY F. CARSON

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research for acceptance, a thesis entitled Development and Reproductive Biology of Three Species of *Solaster* Sea Stars from the Northeast Pacific Ocean submitted by Sally Fraser Carson in partial fulfillment of the requirements for the degree of Master of Science.

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Date..... Sept. 21, 1989.....

ABSTRACT

Solaster stimpsoni, S. dawsoni and S. endeca are sympatric asteroid species commonly found in the waters of Barkley Sound, B.C., Canada. They are broadcast spawners during which the females release large buoyant eggs (0.88-1.00 mm greatest diameter). The embryonic and larval developmental patterns, chronology and larval morphology of all three species are similar. Cleavage is total and equal, forming a wrinkled blastula and later a modified brachiolarian larva which is pelagic and lecithotrophic. Settlement occurs 8-10 days after fertilization. Five rays form initially, and the remaining rays are added in sequence between the first and fifth rays. Metamorphosis is completed 40-50 days after fertilization. The juveniles are 1-1.5 mm in diameter, have their full complement of rays with two pairs of tube feet and one terminal tentacle per ray. Arm number is variable and not distinct for each species. S. endeca is bright orange, while S. stimpsoni and S. dawsoni are both tan and difficult to separate.

The distribution of juveniles in the field and results of settlement experiments in the laboratory indicate that there is no separation of juvenile and adult habitats, or evidence of a juvenile "nursery". In the laboratory, Solaster larvae indicate a preference for Phyllochaetopterus tubes when given a choice with rocks. When the two substrata are presented alone there is no difference in degree of settlement. Juveniles are able to survive on a diet of detritus for 14 months but exhibit little growth.

The reproductive cycle of the three Solaster species is similar as indicated by gonad indices, oocyte size frequencies and natural spawning observations. There is a distinct annual pattern with spawning occurring in February and March. No relationship exists between the gonadal and pyloric caecal index of S. stimpsoni. An overlap in habitat and niche of the three Solaster spp. is indicated by analysis of field distribution and food preference. Although there is no visual evidence of hybridization , cross fertilization and development through to metamorphosis is possible between all pairs of the three species in the laboratory. Reduced survivorship of hybrids as compared to conspecific progeny may

be important in the separation of species in nature. Neither hybrids or conspecific cultures were reared to maturity.

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TABLE OF CONTENTS

Chapter — Page

1. General Introduction 1

Literature Cited 5

2. Development, metamorphosis and substrata selection by three species of Solaster from the Northeast Pacific Ocean.

Introduction 6

Materials and Methods 8

Results

Reproductive system and induction of spawning 11

Embryonic development 13

Larval development 17

Metamorphosis 23

Settlement behavior 30

Juvenile Development 32

Juvenile distribution 32

Discussion

Embryonic and larval development 36

Juvenile distribution 41

Literature Cited 44

3. Reproductive cycles and possible barriers to hybridization in three species of Solaster from the Northeast Pacific Ocean.

Introduction 49

Materials and Methods

Gonad Index 51

Oocyte size frequencies 54

Hybridization experiments 55

TABLE OF CONTENTS (con't)

Chapter Page

3. Reproductive cycles and possible barriers to hybridization in three species of Solaster in the Northeast Pacific Ocean.

Material and Methods

Population survey 55

Results

Pyloric caeca index 56

Gonad index 56

Oocyte size frequency 64

Spawning observations 73

Distribution 73

Hybridization 77

Discussion

Reproductive cycle 83

Hybridization 86

Literature Cited 88

4. General discussion and conclusion 93

5. Appendix

Overview table of chronology of development in three species of Solaster 96

LIST OF TABLES

Table — Page

1. Egg size of Solaster stimpsoni, S. dawsoni, and S. endeca 12
2. Chronology of embryonic development in three Solaster species 16
3. Chronology of larval development in three Solaster species 19
4. Size of larvae and juveniles of Solaster stimpsoni, S. dawsoni, and S. endeca ... 20
5. Chronology of metamorphosis in three Solaster species 29
6. Substratum preference in larval settlement for Solaster species 31
7. Size range of Solaster stimpsoni in various locations 33
8. Size range of Solaster dawsoni in various locations 35
9. Natural spawning observations for Solaster stimpsoni and S. dawsoni 74
10. Distribution of Solaster stimpsoni, S. dawsoni and S. endeca near Bamfield B.C. - Transect survey 75
11. Distribution of Solaster stimpsoni, S. dawsoni and S. endeca near Bamfield B.C. - Diving observations. 76
12. Results of hybridization experiment among Solaster species 78
13. Summary of results of hybridization experiments among the genus Solaster ... 79
14. Chronology of development of Solaster hybrids..... 81
15. Size of larvae and juveniles of Solaster hybrids..... 82

LIST OF FIGURES

Figure Page

1. Photomicrographs of embryonic and larval development of Solaster stimpsoni 14
2. SEM micrographs of metamorphosis of Solaster stimpsoni 22
3. Photomicrographs of early metamorphosis of Solaster stimpsoni with and without attachment to a substratum 24
4. SEM micrographs of metamorphosis and juvenile development of Solaster 27
5. Location of sites from which Solaster was collected for gonad index data in Barkley Sound, B.C., Canada 52
6. Solaster stimpsoni. Monthly mean gonad and pyloric caeca index using total weights, from Barkley Sound, January 1985 to April 1987 57
7. Solaster stimpsoni. Monthly mean gonad indicies using total weights from Bamfield Inlet and Taylor Island areas, January 1985 to April 1987..... 59
8. Phoromicrograph of cross section through ovary of Solaster stimpsoni in April 1985, showing degeneration of large oocytes..... 62
9. Solaster stimpsoni. Seasonal changes in the relative size frequency of oocytes during 1985 65
10. Solaster dawsoni. Seasonal changes in the relative size frequency of oocytes during 1985 67
11. Solaster endeca. Seasonal changes in the relative size frequency of oocytes during 1985 69
12. Photomicrograph of ovary cross section of Solaster stimpsoni during November 1985 71

CHAPTER 1

GENERAL INTRODUCTION

Six asteroid species in the Family Solasteridae occur in the Northeast Pacific Ocean; Solaster stimpsoni, Solaster dawsoni, Solaster endeca, Solaster paxillatus, Crossaster papposus and Lophaster furcilliger. Solaster is a very conspicuous and important genus in the rocky, shallow-subtidal habitats of Barkley Sound on the southwest coast of Vancouver Island. Solaster sea stars are large, fairly abundant, and may be considered important predators as they are specialist feeders on certain asteroids and holothurians. These sea stars are ecologically important due to their abundance and influence on community structure in Barkley Sound; however, there is little information available pertaining to their larval and juvenile stages, or their reproductive biology. All Solaster species are thought to broadcast spawn, live in the same habitat, and to reproduce at the same time of year (Strathmann, 1987).

Solaster stimpsoni is the most abundant of the four species found in Barkley Sound. Its geographic distribution in the north Pacific extends from Alaska, south to California and Japan (Furlong and Pill, 1970; Lambert, 1981). Its vertical distribution ranges from the intertidal zone to 610 m, but highest densities are found in the shallow subtidal zone on boulder substrata where sea cucumbers, its preferred food, are dense. The radius (R), measured from the center of the disc to the tip of the longest arm, can reach 230 mm. It has 8-11 arms and is usually bright orange with a dark blue stripe running down the dorsal surface of each arm.

Engstrom (1974) reported that 99% of the diet of S. stimpsoni in Puget Sound consists of Cucumaria lubrica. The rest consists of Cucumaria miniata and Eupentacta quinquesemita. In Barkley Sound, C. miniata is more abundant than C. lubrica, and appears to be the major component of its diet. Mauzey et al. (1968) found that, in the laboratory, S. stimpsoni preferred C. miniata to C. lubrica. To feed, they evert the cardiac stomach over an individual prey item and draw it inside after partial digestion.

Solaster dawsoni, like S. stimpsoni, is restricted to the north Pacific, ranging from Monterey, California north to the Arctic Ocean and south to the Yellow Sea of Asia (Verrill, 1914; Lambert, 1981). Its vertical distribution extends from the intertidal zone to 420 m. It is generally smaller than S. stimpsoni, with a radius of up to 200 mm, but the central disc is proportionately larger, and usually with more arms (10 to 13). The colour is uniform, ranging from tan to reddish brown.

S. dawsoni is a tertiary consumer feeding mainly on S. stimpsoni and other sea stars, such as Pycnopodia helianthoides, Leptasterias hexactis, Evasterias troscheli Pisaster ochraceus, Pisaster brevispinus, Dermasterias imbricata, Crossaster papposus, Mediaster , Henricia leviuscula and occasionally on holothurians (Mauzey et al, 1968; Engstrom, 1974). S. dawsoni will attack conspecifics, but such animals can escape often by bending their arms back and pushing the attacker off, while rapidly moving forward (10 cm/min.) (Mauzey et al, 1968). Adults of S. dawsoni require 5 days to capture and consume each prey item once it is located (Birkeland, 1974). To capture their prey, the sea star moves on to the dorsal surface and entwines its rays around those of the asteroid. The central disc of the prey is ingested first and the rays follow.

Solaster endeca is circumboreal in distribution, occurring as far south as Great Britain and Cape Cod in the north Atlantic (Verrill, 1914), the Arctic Ocean, and south to Puget Sound and East Siberia in the north Pacific (Engstrom, 1974; Lambert, 1981). It is less common than the other two species. It's vertical range extends from the intertidal zone to 475 meters. It is easily distinguished from the other two species on the basis of shape, as it has a broader disc and shorter, sharply tapered arms. It is generally smaller (average R in B.C. waters is 90 mm) and has fewer arms (7-10). It is bright yellow-orange, occasionally with a blue or reddish purple stripe down the dorsal surface of each arm. The diet of S. endeca is broader than that of S. stimpsoni but consists of basically similar items; C. lubrica, C. miniata, Thyone benti, bryozoans, ectoprocts and ascidians (Lambert, 1981, Engstrom, 1974).

S. paxillatus is rare and is only found in deeper water (> 25 m). Its distribution ranges from the Bering Sea, south to Northern Oregon and Yokohama, Japan (Lambert, 1981). It is bright yellow-orange, with 8-12 arms and a radius of up to 190 mm. Little information has been collected on its feeding behavior, but it appears to prefer asteroids and holothurians. This species was not included in this study due to the limited number of specimens found.

The main cause of mortality of S. stimpsoni appears to be S. dawsoni, although like conspecifics they exhibit a dramatic and successful escape response from S. dawsoni. This behavior has not been observed in S. endeca (Mauzey et al, 1968). From both field and lab observations, Engstrom (1974) noted that S. endeca is much less active than S. stimpsoni while S. dawsoni is a very mobile species (Engstrom, 1974; Birkeland, 1974). The low density of S. dawsoni may be explained by the time spent and the distance covered by these stars in locating their next meal (Engstrom, 1974). Their food may not be concentrated in one area, so they, in turn, are not found at high densities. Their tendency to eat conspecifics may also promote spacing.

Other mortality sources reported by Engstrom (1974) for S. stimpsoni in Puget Sound include a parasitic green algae, Diogenes sp., and an endoparasitic ascothoracian barnacle, Dendrogaster. A small portion of the animals in Barkley Sound are infected with this algae, but no evidence of the barnacle was observed in this study. Engstrom (1974) also described a snail, Fusitriton, which is rare in Barkley Sound, and an amphipod, Parapleustes, to be further causes of mortality in Solaster. A commensal worm of the genus Arctenoe is frequently found in the ambulacral groove of all species of Solaster.

Information on growth rates for sea stars is limited. Engstrom (1974) estimated on the basis of laboratory growth rates and a computer model (Febens, 1965) that S. stimpsoni reaches maturity (100 g) in 4 - 4 1/2 years. Assuming that steady, deterministic growth is maintained, Engstrom's (1974) field data suggests ages of 10-15 years with 9.6 % of the population greater than 10 years old.

Knowledge of what occurs during transition from a post metamorphic juvenile to an adult is lacking for most asteroid species. Juveniles are not commonly observed in the field, possibly due to their small size, cryptic appearance, the separation of adult and juvenile habitats, or because of very low and sporadic recruitment. Observations of juveniles in the laboratory have not met with much success due to low survival. In Puget Sound, Washington, Birkeland et al. (1971) noted that small Solaster stars were found on beds of Phyllochaetopterus worm tubes.

This study was conducted at the Bamfield Marine Station on Barkley Sound, Vancouver Island, British Columbia, Canada and was designed to address the following points: (1) description and comparison of the embryonic, larval and juvenile development for S. stimpsoni, S. dawsoni and S. endeca; (2) determination and comparison of the reproductive cycles for all three species; (3) distribution of adults and juveniles for all three species; (4) cross fertilization experiments and speculation of how reproductive isolation between the three species is maintained.

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CHAPTER 2

Development, Metamorphosis and Substrata Selection by Three Species of Solaster from the Northeast Pacific Ocean.

INTRODUCTION

Three species of the asteroid genus Solaster (Order Spinulosida, Family Solasteridae) commonly occur in the northeast Pacific Ocean: Solaster stimpsoni Verrill 1880, Solaster dawsoni Verrill 1880 and Solaster endeca Linnaeus 1771. A fourth species, Solaster paxillatus Sladen 1889, is also found in the northeast Pacific Ocean but is relatively rare.

A lecithotrophic mode of development for S. endeca from the northeast Atlantic Ocean was described by Gemmill (1912). Crossaster papposus, the only other species in the Family Solasteridae (Moore, 1966) for which development has been described (Gemmill, 1920), has a lecithotrophic mode of development similar to that described for S. endeca. The development and metamorphosis of S. stimpsoni and S. dawsoni have not been described, but it has been noted that they are broadcast spawners which release large yolky eggs in early spring (Strathmann, 1987).

Lecithotrophic development, in which all nutrients needed for larval life are stored in the egg, is usually associated with large eggs, low fecundity and a shorter pelagic period than planktotrophic development. Lecithotrophic development is found in 15% of echinoderms (Grahame and Branch, 1985), 44% (42/95) of all asteroids and 66% (19/29) of asteroids in the Order Spinulosida known to have pelagic larvae (Emlet et al., 1987).

S. stimpsoni, S. dawsoni and S. endeca are sympatric and occur on rocky substrata in the shallow subtidal. They are secondary and tertiary consumers, feeding on asteroids and holothurians, and are considered to be principle members of subtidal food chains (Mauzey et al, 1968; Engstrom, 1974). Despite their ecological significance, we know very little about the larval and juvenile stages of their life history. A detailed description of

developmental patterns of these co-occurring congeners is necessary before the question of recruitment, reproductive isolation and ecological effect of Solaster on the benthic community can be addressed. Settlement behavior of larvae can affect distribution and post metamorphic survival of the species.

This chapter reports detailed observations on the larval development of three Solaster species. External morphology, chronology and size of the various developmental stages are compared between the three species of Solaster, with Gemmill's (1912) description of S. endeca, among the Family Solasteridae and with other lecithotrophic asteroids. I have redescribed the development of S. endeca to facilitate comparison between the three species and to compare development of S. endeca in the Atlantic and Pacific Oceans. Gemmill (1912) did not describe some developmental stages, nor did he record culture temperature or larval size. Some information is also presented on the settlement behavior of larvae and on the development and distribution of juveniles.

MATERIALS AND METHODS

All three species of Solaster were collected using SCUBA from various locations in the southwest part of Barkley Sound on the west coast of Vancouver Island, British Columbia, Canada (48°49'N, 125°08'W). Collections were made at depths of 4-25 m, from February to April, 1985 and 1986. Sex was determined by making a small incision in the disc at the interradius and removing a small piece of gonad.

To induce oocyte maturation and spawning, females were injected with the hormone 1-methyladenine (1-MA), a natural inducer of spawning and maturation found in the ovary (Kanatani et al, 1969), from late February to mid April. Three to five ml of 1-MA ($1x10^{-4}$ M) were injected into the perivisceral coelom of the disc through the mouth and approximately 1 ml was injected into the body cavity at the base of each arm. Sperm suspensions were prepared by placing a small piece of testis in 200-300 ml of filtered (100 um) sea water.

Spawned eggs were collected as they floated to the surface and were rinsed in filtered sea water 3-4 times. Sperm were checked for motility and then 1 to 3 drops of sperm suspension were added to the dish (400-600 ml) with the eggs. The same male was used to fertilize all the eggs from one female, but a variety of males were used to fertilize eggs from different females. Eggs were usually fertilizable if they were regular in shape, the jelly coat had been shed and the membrane of the germinal vesicle had broken down. The majority (>90%) of these eggs were fertilized (as indicated by formation of the fertilization membrane), but only 38-71% hatched successfully. It is unlikely that polyspermy was the cause of this early mortality, because developmental success did not vary consistently with tconcentration of sperm.

Fertilized eggs were placed in glass culture dishes (400-600 ml) at a concentration of 500-1000 eggs/dish. Culture dishes were placed on a water table with running sea water at a temperature of 10-12° C. Embryos were transferred with a pipette to clean filtered (100 um) sea water daily until the larvae hatched from the fertilization membrane, when the

water was changed every second day. If there were any signs of bacterial infection, streptomycin (250 mg/l) was added to the culture (Strathmann, 1987).

Developmental times for each larval stage were recorded in three ways: (1) the minimum time it took the majority (>50%) of individuals in a culture to reach a given stage, (2) the minimum time it took the majority of individuals in a culture, which successfully metamorphosed, to reach a given stage, and (3) the mean time for all cultures to reach a given stage. All three times were recorded because there was a large amount of variation in rate of development within and between cultures. The minimum time may be biased towards exceptionally fast developers, and the mean time may be biased towards extremely slow developers, which may not have completed metamorphosis. The mean time of those cultures in which some individuals completed metamorphosis is probably the most reliable time schedule and is recorded in Appendix 1, but because such a small percentage of cultures completed metamorphosis, the number of replicate cultures is limited. Times for every developmental stage were not recorded for each culture, thus the average developmental time for each stage was calculated for a different number of observations. This, along with variation in developmental times between cultures, explains why the sequence of events appears to be reversed in certain cases.

Measurements of living embryos were made with an ocular micrometer. Some eggs were placed in Proto Slo (Carolina Biological Co.) to facilitate turning the eggs on their sides long enough to measure the animal-vegetal axis. Egg volume was calculated using the formula $v=4/3\pi(D1/2)(D2/2)(D3/2)$. Greatest length and width of larvae at various stages were measured. Average size (length x width) for a developmental stage was determined for individual cultures. The number of larvae measured per culture varied, so to weigh each culture evenly, the average size for a culture was used to obtain a mean size for that stage of development.

Embryos of different stages were fixed for scanning electron microscopy (SEM). Larvae were rinsed in millipore filtered sea water three times to remove any foreign material

from the larvae, and some were then anesthetized with 7% $MgCl_2$ in sea water. They were then fixed in 2-4% osmium (in sea water) for 10 minutes, rinsed with sea water, then distilled water, dehydrated to 100% alcohol, subject to critical point drying with CO_2 and then mounted on stubs and coated with gold before examination with a scanning electron microscope.

Tubes of the polychaete, Phyllochaetopterus prolifica or small rocks were provided as substrata for larvae to settle on, as these were the substrata that juveniles were found on in the field. The process of metamorphosis was then easy to observe as the substratum could be removed from the dish and viewed from any angle. When metamorphosis was complete, juveniles were placed in containers with a mesh bottom, in a flow-through sea water system, with the substratum they had settled on.

Settlement experiments were conducted to see if the larvae required a substratum for metamorphosis and to learn whether they exhibited any preference for settlement on rocks, Phyllochaetopterus tubes or the dish surface. Settlement experiments were performed in glass or plastic, 30 ml dishes, 5 cm in diameter. Ten healthy, well developed, brachiolarian larvae were placed in each dish. The dishes each contained one of four substrata conditions: (a) 3, 1-2 cm pieces of Phyllochaetopterus tubes, (b) 2 rocks, 1 cm in diameter, (c) 2, 1-2 cm pieces of Phyllochaetopterus tubes and 1 or 2 rocks, 0.5-1 cm in diameter, (d) control, no additional substrata added to the dish. These dishes were placed in an incubator, at 10-12° C, for 5 days and experiments were checked daily, the number of larvae which had settled and the substratum choice was recorded and half the water in each dish was changed. A two way analysis of variance by ranks was used to test for differences between species, between substratum conditions and to see if there was any interaction between species and condition (Zar, 1974).

To determine whether adults and juveniles were occupying the same habitat, populations were surveyed both qualitatively and quantitatively. Size (R in mm) of all Solaster encountered when diving at different locations was recorded.

RESULTS

Reproductive System and Induction of Spawning

All three species of Solaster are gonochoric. The gonad, a cluster of cylindrical or simply branched tubes, is found in pairs at the base of each ray. The gonads open, via the gonoducts, to the aboral surface at each interradius. The ovary was easily distinguished from the creamy white testis, as it reflected the colour of the ova; olive-green in S. stimpsoni, brownish-green in S. dawsoni and orange-red in S. endeca. In all three species, colouration of eggs was darker in the upper half (when floating in water), the animal pole. The lower half, the vegetal pole, seemed to have greater specific gravity so that the egg rights itself almost at once if if were disturbed.

The mean egg volume for S. stimpsoni was 0.406 mm^3 (McEdward and Carson, 1987), 0.376 mm^3 for S. dawsoni and 0.285 mm^3 for S. endeca (Table 1). The eggs are oblate spheroids in which the animal-vegetal axis is significantly shorter than equatorial diameter, although there is no consistent relationship between the two (McEdward and Carson, 1987). The mean of the greatest equatorial diameter and the mean of the animal-vegetal axis are also reported for comparison of egg size with that of other asteroids found in the literature (Table 1). Formation of polar bodies was not observed through a dissecting microscope, although this may have occurred before the eggs were collected.

Spawning took anywhere from 8-12 hours after injection of 1-MA. The time between injection of the 1-MA and release of ova is similar to that observed for the lecithotrophic asteroid, Ctenodiscus criptatus at 10°C (Shick et al, 1981), but much longer than is recorded for other asteroids with planktotrophic development (Strathmann,1987; Fraser et al, 1981). Natural spawning was observed in the laboratory in three S. stimpsoni females. Eggs from these spawns were fertilized and two of the three cultures hatched (66%), while only one culture completed metamorphosis (33%). Only 35% of cultures from 1-MA induced spawns hatched and only 4% completed metamorphosis. Developmental success appears to be higher with natural spawns.

Table 1 - Egg Size of S. stimpsoni, S. dawsoni and S. endeca

Species	Volume (mm^3)			Diameter (mm)				
				equatorial			animal-vegetal	
	N	$\bar{X}$	(SD)	N	$\bar{X}$	(SD)	$\bar{X}$	(SD)
S. stimpsoni	658	0.41	(0.04)	200	1.00	(0.04)	0.80	(0.05)
S. dawsoni	36	0.38	(0.03)	36	1.00	(0.03)	0.73	(0.04)
S. endeca	24	0.29	(0.02)	24	0.88	(0.03)	0.71	(0.03)

Embryonic Development

Fertilization occurred soon after sperm were added to the eggs. The fertilization membrane started to lift at one point, presumably the point of sperm penetration, and gradually lifted to equally surround the egg and form a perivitelline space of 0.035-0.070 mm. In S. stimpsoni, the minimum time from addition of sperm until most of ova in a culture formed a fertilization membrane was 0.5 hr., in S. dawsoni it was 3.5 hr and in S. endeca it was 1 hr. To eliminate this variation, developmental times were recorded as time elapsed since formation of the fertilization membrane. There was at least 90% fertilization in cultures that successfully metamorphosed.

Cleavage, which began soon after formation of the fertilization membrane, was total, radial and usually equal. The opaque coloration of the eggs made only external changes visible and individual cells were often hard to distinguish especially in the red embryos of S. endeca. The first cleavage was meridional, along the animal vegetal axis, the second, meridional and perpendicular to the first (Fig. 1a), and the third equatorial, and separating the animal and vegetal halves. Cleavage continued, to form a morula, in which the cells were usually equal in size. Developmental times for these early cleavage stages corresponded in all three species (Table 2).

The early blastula appeared smooth and resembleed the newly fertilized egg. Then, numerous furrows formed on its external surface, giving the blastula a finely lobulated or wrinkled appearance (Fig. 1b). The wrinkled blastula originally resembled the 16 or 32 cell stage but gradually some of its furrows disappeared and others deepened to resemble the earlier cleavage stages. The blastopore forms as an enlargement of one of the furrows.

Most of the surface furrows of the blastula disappeared before hatching occurred: 1.5-2 days in S. stimpsoni and S. dawsoni, and 2-2.5 days in S. endeca (Table 2). Mortality of cultures was highest during this stage. As the wrinkles decreased in number, many (>90% in cultures that did not reach metamorphosis, and <50% in cultures that did metamorphose) embryos became covered with brown dots and then turned whitish green.

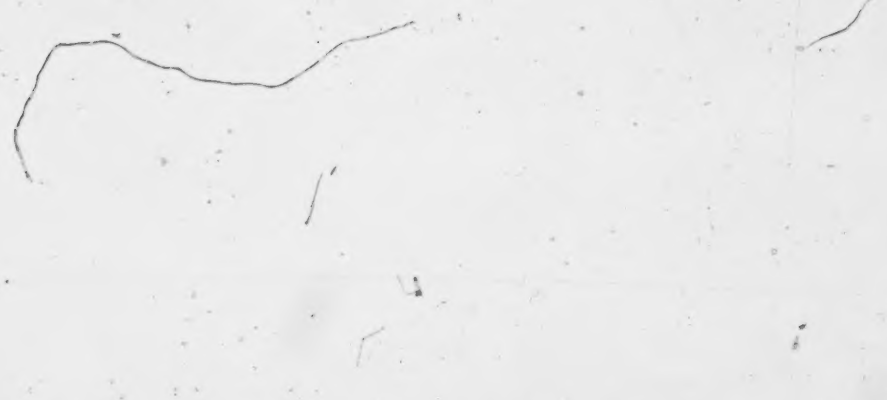


Figure 1 - Photomicrographs of embryonic and larval development of S. stimpsoni.

A. 4 cell stage.

B. Wrinkled blastula.

C. Two cone shaped gastrulae, arrow indicating the blastopore.

D. Brachiolarian larva showing pre-oral lobe (p), arms (a), sucker (s) and an arrow indicates the hydroporic opening.

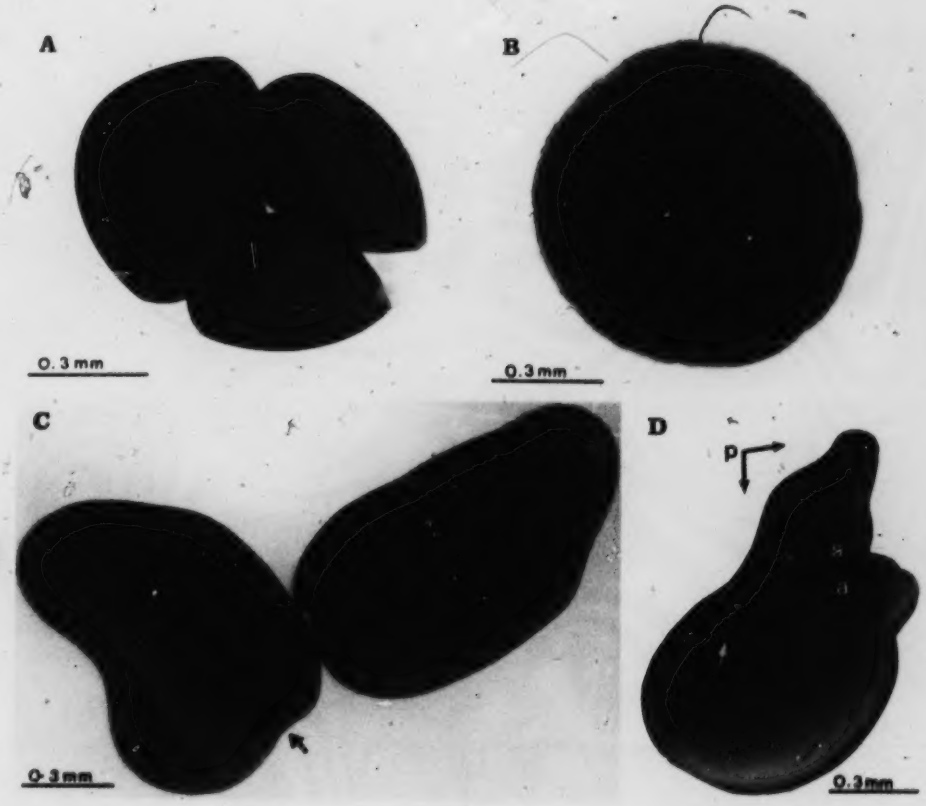

A
0.3 mm
B
0.3 mm
C
0.3 mm
D
p
0.3 mm

Table 2 - Chronology of Embryonic Development in Three Solaster Species (hours)
10 - 12 °C

Stage of Development	S. stimpsoni			S. dawsoni			S. endeca		
	Min.1	Min.2	$\bar{X}$	Min.1	Min.2	$\bar{X}$	Min.1	Min.2	$\bar{X}$
cleavage furrow	1		[4]	1		[1]	2		[2]
4 - cell	3	(3)	[3.5]	3	(3)	[3.5]	3	(3)	[3.5]
8 - cell	3	(3)	[8.5]	3	(3)	[4]	4	(5)	[6.5]
32 - cell	7	(16)	[10]	10		[10.5]	7	(7.5)	[9.5]
64 - cell	7		[11.5]	6.5		[11]	11		[13]
morula	12.5	(16)	[18]	12.5	(15.5)	[16]	11	(11)	[19.5]
16 - cell	5.5	(5.5)	[8]	3.5	(3.5)	[4]	2	(2)	[7]
early blastula	12.5	(18)	[21.5]	15.5	(17.5)	[18]	19	(19)	[28]
wrinkled blastula	19.5	(20.5)	[26]	19.5	(24)	[29.5]	25	(25)	[35]
hatching	28.5		[51]	37.5	(37.5)	[50]	46	(53)	[66]

Min.1 - minimum time (h) it took most individuals in a culture to reach that stage.
Min.2 - minimum time (h) it took most individuals in a culture that metamorphosed to reach that stage.
$\bar{X}$ - average time (h) it took most individuals in a culture to reach that stage.

The furrows completely disappeared and the fertilization membrane ruptured (hatching), releasing the soupy contents. In some cases, only part of the embryo went through this process and when the fertilization membrane ruptured, an abnormal spinning gastrula, often half the normal size, was released. These small gastrulae never completed metamorphosis. The majority of larvae in a culture either died at this point, or went on to metamorphose successfully. With S. stimpsoni 38% (12/32) of the cultures hatched successfully, 62% (8/13) in S. endeca, and 71% (5/7) in S. dawsoni.

Larval Development

Once free from the fertilization membrane, the buoyant gastrula spins continuously around its vertical axis by ciliary beating. The direction of rotation of gastrulae, as viewed from the animal pole, was to the counter clockwise in 59% (196/331) of S. stimpsoni and S. endeca (197/335) and about 50% in S. dawsoni. The direction of rotation of individual larvae appeared to be constant and to result in little forward motion. The blastopore is a conspicuous circular opening in the middle of the distinctly flattened lower surface. Development is not accompanied by change in size until elongation of the gastrula along the vertical axis (Fig. 1c). The blastopore is still present, its position defining the posterior end of the embryo, but it is slowly decreasing in size. At this time, the density of the embryo appears to increase, causing it to move to the bottom of the water column. The embryo remains in a vertical or slanting position with its narrow anterior end uppermost. Embryos were still spinning with a higher percentage rotating counter clockwise: 62% (139/224) of S. stimpsoni and 70% (158/227) of S. endeca. Soon after reaching the bottom, embryos are capable of vertical and horizontal movement. If not rotating around their vertical axis, they are moving forward on their sides. Embryos of all three species moved posterior end first, but occasionally they tumbled, end over end. Most of embryos remained near the bottom of the culture dish and often associated with other larvae, to form dense

aggregations. All three species were almost synchronous in the formation of elongated gastrulae and in movement to the bottom (Table 3).

The embryo becomes a larvae with the development of the larval structures; pre-oral lobe, arms and sucker. As the pre-oral lobe begins to differentiate, a number of dimples and furrows develop. Development of the pre-oral lobe involves a constriction 1/3 down the lenght of the body from the anterior end and a slight flexion was noted (Fig. 1d). An indentation formed at the point of flexion marks the location of the sucker and defines the ventral surface of the larva. S. stimpsoni larvae show their greatest increase in size at this point (Table 4). About mid way in length of the larva on its right dorsal surface, an indentation forms around a small pore (hydroporic opening). This is the first interference to the external bilateral symmetry of the larvae. Three brachiolarian arms develop on the ventral surface of the pre-oral lobe. They begin as small bumps, one anterior, and two lateral, surrounding the point of flexion. As the arm bumps gradually lengthen, their tips become slightly adhesive and the cilia in this area become sparse (Figs. 2a,2b). The sucker develops as a round, slightly elevated disc between the three arms. This area darkens, changes texture slightly and most of its cilia are lost (Fig. 2a). The larva, with three arms and a sucker is now a well developed, modified, brachiolaria (Fig. 2a, Table 3). Development of arms, sucker and hydroporic opening in larvae of S. stimpsoni and S. endeca occur within 1-2 days of each other (Table 3). Sucker formation in S. dawsoni takes longer than formation of arms or hydroporic opening (Table 3). The brachiolaria of all three species are very similar in morphology and range in size from 0.97 x 1.58 mm in S. stimpsoni to 0.98 x 1.50 mm in S. endeca (Table 4).

The brachiolarian arms are spread apart, exposing the sucker as the larva swims. This could be asignal that the larva is competent, and ready to settle and metamorphose. The larva is also capable of pulling the arms together and hiding the sucker. The glandular tips of the brachiolarian arms (Fig. 2b) seem to be important in detecting or in adhering to a substratum. The arms appear to be used for temporary attachment and if metamorphosis is

Table 3 - Chronology of Larval Development in Three Solaster Species (days)
10 - 12 °C

Stage of Development	S. stimpsoni			S. dawsoni			S. endeca		
	Min.1	Min.2	$\bar{X}$	Min.1	Min.2	$\bar{X}$	Min.1	Min.2	$\bar{X}$
cone-shaped gastrula	3.5	(3.5)	[6]	3.5	(4)	[4]	4	(4)	[5.5]
sink to bottom	5	(5)	[7]	4	(4)	[6]	5	(6.5)	[5.5]
pre-oral lobe form	5	(5)	[5.5]	4	(4)	[4.5]	7		[7]
brachiolarian arms develop	7	(7)	[14]	5	(5)	[12]	6	(6)	[14.5]
sucker develop	7	(7)	[14.5]	9	(10)	[13]	6.5	(9)	[13.3]
hydroporic opening form	7	(7)	[11]	5	(5)	[6]	7	(7)	[17]
attachment to substratum	9	(9)	[22]	8	(8)	[13]	11	(11)	[24.5]

Min.1 - minimum time (d) it took most individuals in a culture to reach that stage.
Min.2 - minimum time that it took the majority of individuals in a culture that metamorphosed to reach that stage.
$\bar{X}$ - average time that it took the majority of individuals in a culture to reach that stage.

Table 4 - Size of Larvae and Juveniles of S. stimpsoni, S. dawsoni and S. endeca

Stage of Development	Size [$\bar{X}$ Length (range) x $\bar{X}$ Width (range)] mm								
	S. stimpsoni			S. dawsoni			S. endeca		
	N	L	W	N	L	W	N	L	W
early brachiolarian larvae	23	1.6 (1.3-1.6)	1.0 (0.8-1.2)	15	1.4 (1.3-1.6)	0.9 (0.8-1.1)	21	1.1 (0.9-1.4)	0.7 (0.6-0.9)
brachiolarian larvae	53	1.6 (1.2-2.1)	1.0 (0.8-1.4)	24	1.5 (1.3-1.7)	0.9 (0.7-1.0)	2	1.5 (1.1-1.9)	1.0 (0.8-1.2)
Juv. 5 hydrocoel pouches	1	1.3	1.2	1	1.1	1.1	1	1.4	1.0
Juv. 6 sets tube feet	19	1.3 (1.2-1.4)	1.2 (1.1-1.3)	14	1.3 (1.1-1.5)	1.2 (1.1-1.5)	6	1.2 (1.1-1.4)	1.0 (0.9-1.1)
Juv. 9-12 sets tube feet	16	1.5 (1.3-1.3)	1.4 (1.2-1.6)	6	1.3 (1.2-1.4)	1.3 (1.1-1.3)	2	1.4 (1.4-1.5)	1.4
Juv. with open mouth	35	1.5 (1.2-1.8)	1.4 (1.2-1.7)	54	1.6 (1.4-1.9)	1.5 (1.2-1.8)	32	1.5 (1.2-1.8)	1.3 (0.9-1.6)
14 month old juvenile		1.7 (1.4-2.0)	1.6 (1.4-1.8)		1.6 (1.2-1.9)	1.6 (1.3-2.2)		1.6 (1.2-1.9)	1.5 (1.2-1.8)

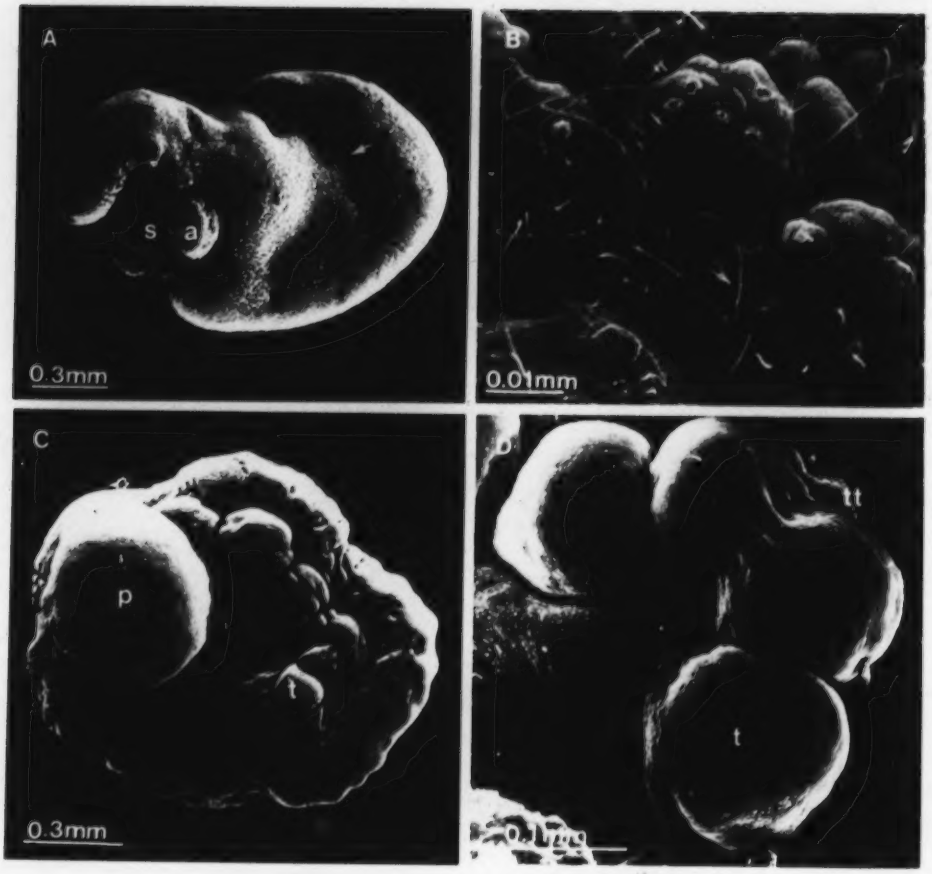
Figure 2 - SEM micrographs of metamorphosis of S. stimpsoni

A. Brachiolarian larva with well developed arms (a) and sucker (s). Indentations visible on the left side of the larval body indicate the sites of hydrocoel pouches of the water vascular system (arrow).

B. Surface of brachiolarian arm showing papillae and cilia.

C. Metamorphosing larva showing degenerating pre-oral lobe (p) and the hydrocoel pouches and the begining of tube foot differentiation (t).

D. Division of hydrocoel pouch to form two pairs of tube feet (t) and the terminal tentacle (tt).


A
s
a
0.3mm
B
0.01mm
C
p
0.3mm
tt
t

to follow, the larvae spread their arms and adhere to the substratum with the attachment disc. The attachment disc appears to be covered with secretory cells. Well developed S. stimpsoni larvae would attached to forceps when probed, which suggests that there may be a sticky secretion on the arms or sucker. Attachment occurs first in S. dawsoni (8 d), about a day or so later in S. stimpsoni (9 d) and a couple of days after that in S. endeca (11 d) (Table 3).

Metamorphosis

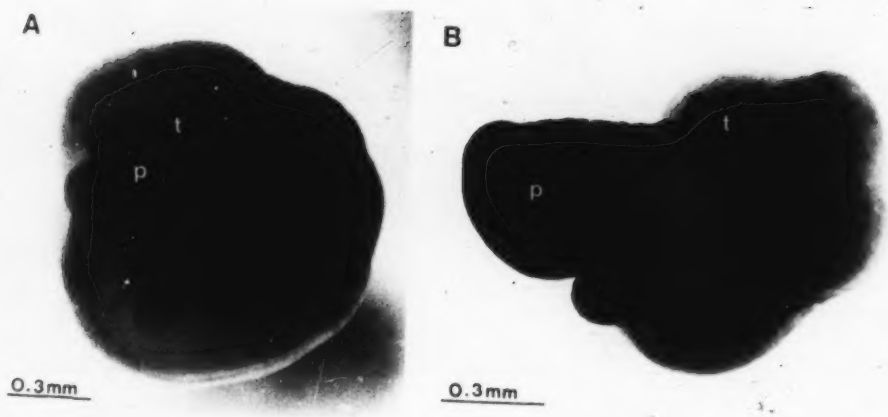
When a larva initially attaches, its body projects from the substratum. The posterior end becomes laterally flattened and disc-shaped, and slightly concave on the left side and convex on the right . Flexion and torsion of the pre-oral lobe follow. The left side of the larval body bends towards the pre-oral lobe and torsion of its neck to the right brings its dorsal surface towards the left side of the body (Fig. 2c). The pre-oral lobe is now perpendicular to the left surface of the metamorphosing larva and to the substratum. This position is similar to that described by Gemmill for larvae of S. endeca (1912) and C. papposus (1920). This change in body form can occur before settlement and does not seem to depend on attachment. Flexion and torsion of the pre-oral lobe may occur slightly before sucker fixation in larvae of S. dawsoni, a pattern also described in those of C. papposus (Gemmill, 1920). In general, the pre-oral lobe seemed to be absorbed more quickly when attachment on a substratum preceded metamorphosis (Fig. 3a), than when metamorphosis occurred without attachment (Fig. 3b).

The left side of the larva differentiates into the oral surface of the juvenile, and the right sideits aboral surface. The water ring, a horse-shoe shaped indentation, which opens towards the pre-oral lobe in the center of the left side, precedes development of oral structures (Fig. 2a). Five radial canals and single lobular swellings develop on the outer side of the ring. These are the hydrocoel pouches and the beginnings of the tube feet (Figs. 2c,2d,3a,3b). The center of the water ring is where the mouth and stomach develop. Each

Figure 3 - Photomicrographs of early metamorphosis of S. stimpsoni.

A. Resorption of pre-oral lobe (p) and development of tube feet (t) after attachment to substratum.

B. Development of tube feet in a larvae that did not attach to a substratum. Note that the pre-oral lobe (p) is larger than in A. although the tube feet (t) are much more developed.


A
t
p
0.3mm
B
t
p
0.3mm

hydrocoel differentiates into four lobes which will form the primary podia; two pairs of tube feet (Fig. 2d). The terminal tentacle develops soon after. The size of juveniles with 6 sets of tube feet ranged from 1.0-1.29 mm (Table 4) with those of S. stimpsoni largest, and of S. endeca smallest.

Development of subsequent arms follows a definite pattern. The first arm, as viewed from the oral surface is to the right of the pre-oral lobe, and additional arms are usually added in sequence, to the left of the pre-oral lobe. In most larvae (>90%) the pre-oral lobe was absorbed to the left of the first set of tube feet. Eye spots develop below the terminal tentacle following its formation. The central area of the oral surface, where the mouth will form, is often raised. In S. stimpsoni, the sixth and seventh sets of tube feet form at almost the same time (18 d). The eighth, ninth and tenth sets form together about nine days later (27 d) (Fig. 4a, Table 5). The pre-oral lobe has not completely withdrawn until almost 20 days after the larva has its full complement of rays (Table 5). In S. dawsoni the sixth to ninth sets of tube feet form relatively soon after each other but the tenth set does not develop until resorption of the pre-oral lobe and loss of attachment (Table 5). The eleventh and twelfth sets may form after the mouth is open. The rays of S. endeca form in the same sequence and the pre-oral lobe is not resorbed until the last (usually the ninth) set of tube feet is formed. Along with resorption of the pre-oral lobe, comes detachment from the substratum. Thus S. stimpsoni and S. endeca are not capable of extended movement until all rays have formed, but S. dawsoni may be mobile after the appearance of the ninth set of tube feet. Some movement may occur before detachment, so the pre-oral lobe is stretched and resembles an umbilical cord (Chia, 1968) prior to resorption. There is an increase in size, of up to 0.5 mm, as the number of arms increase in all three species (Table 4). When juveniles of each species have developed their full complement of rays, the juveniles of S. stimpsoni and S. endeca are comparable in size (1.36-1.47 mm) and S. dawsoni are slightly smaller (1.3-1.27 mm) (Table 4). Thus size does not seem to relate to arm number.

Figure 4 - SEM micrographs of metamorphosis and juvenile development in Solaster spp.

A. Resorption of the pre-oral lobe (p) in S. stimpsoni.

B. Newly metamorphosed S. stimpsoni juvenile.

C. Newly metamorphosed S. dawsoni juvenile.

D. Newly metamorphosed S. endeca juvenile.

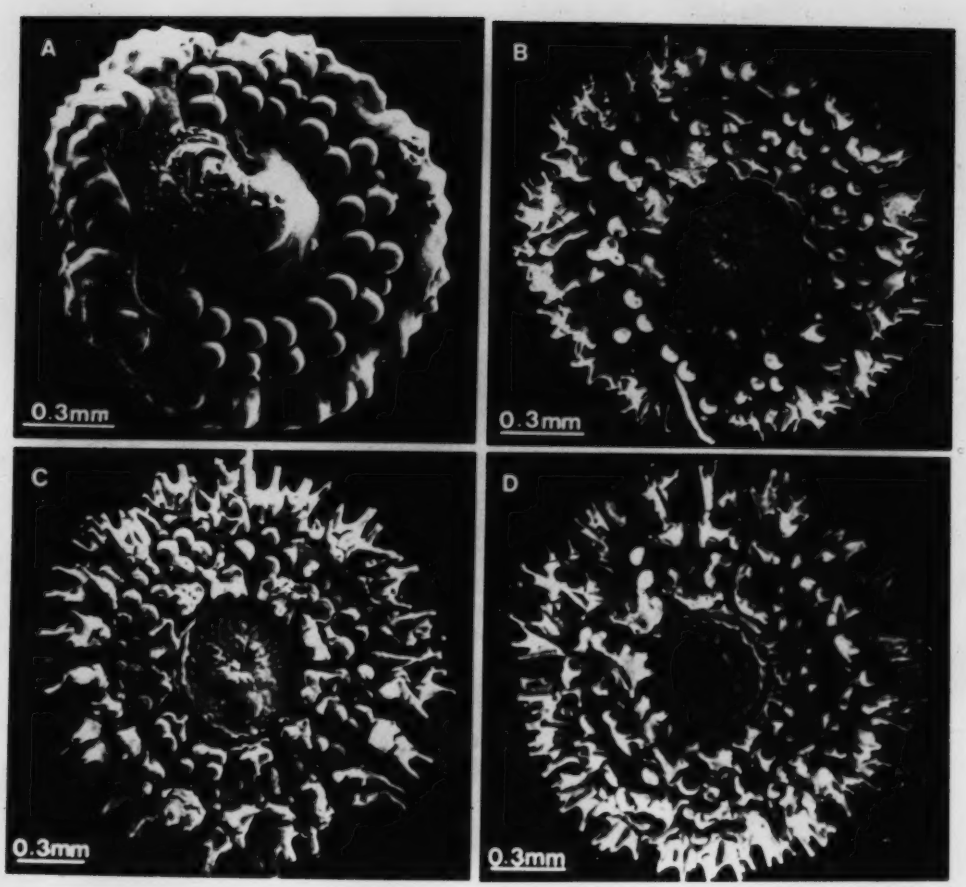

A
0.3mm
B
0.3mm
C
0.3mm
D
0.3mm

Table 5 - Chronology of Metamorphosis in Three Solaster Species (days)
10 - 12 °C

Stage of Development	S. stimpsoni			S. dawsoni			S. endeca		
	Min.1	Min.2	$\bar{X}$	Min.1	Min.2	$\bar{X}$	Min.1	Min.2	$\bar{X}$
turning of larval body	17	(17)	[22]	5	(26)	[16]	12	(12)	[12]
Juv.- 5 hydrocoel pouches	17	(17)	[19]	13	(13)	[13]	12	(12)	[12]
Juv.- 5 sets tube feet	20	(20)	[20]	20	(20)	[22]	13	(13)	[13]
Juv.- 6 sets tube feet	18	(18)	[19]	23	(23)	[23]	22	(22)	[22]
Juv.- 7 sets tube feet	18	(18)	[18]	21	(21)	[22]	15	(15)	[19]
Juv.- 8 sets tube feet	27	(27)	[27]	20	(20)	[22]	20	(20)	[26]
Juv.- 9 sets tube feet	27	(27)	[27]	23	(23)	[26]			
Juv.- 10 sets tube feet	27	(27)	[27]	27		[39]			
Juv.- attachment lost	45	(45)	[50]	24	(24)	[37]	22	(22)	[32]
Juv. with spines	21	(21)	[21]	21	(21)	[27]	48	(48)	[48]
Juv. with mouth open	40	(40)	[46]	43	(43)	[50]	49	(49)	[49]
Juv.- 11 sets tube feet				58	(58)	[58]			
Juv.- 12 sets tube feet				48	(48)	[53]			

Min.1 - minimum time (d) it took most individuals in a culture to reach that stage.
Min.2 - minimum time (d) it took most individuals in a culture that metamorphosed to reach that stage.
$\bar{X}$ - average time (d) it took most individuals in a culture to reach that stage.

As tube feet develop on the oral surface, notches around the edge of the aboral surface become visible, although individual arms cannot be distinguished. One large notch stands out and corresponds to the position of the pre-oral lobe on the oral surface. Spines are also forming on the dorsal surface of Solaster juveniles at this time, although they may only appear as small bumps. Actual spines differentiate at approximately the same time in juveniles of S. stimpsoni and S. dawsoni (21 d) and somewhat later in those of S. endeca (48 d) (Table 5). Cilia are sparsely distributed between the spines on the aboral surface.

The mouth forms at about the same time in all three species (40-50 d) (Table 5). S. endeca has anywhere from 7-11 arms (usually 9), S. stimpsoni has 8-12 arms (usually 10) and S. dawsoni has 9-13 arms (usually 11-12) (Figs. 4b,c,d). Metamorphosis is usually complete at this point and juveniles of all three species have formed and are virtually equal in size, S. dawsoni being largest and S. endeca smallest (Table 4). The anus may not open until 14-16 days after formation of the mouth.

Settlement Behavior

In all three species of Solaster, settlement seems to be induced by presence of a proper substratum. This is indicated by the low degree of settlement in dishes where no additional substratum was present (Table 6). Settlement did occur on the glass surface occasionally and it should be noted that after 5 days, the surface of a dish may be covered with a primary film and that may be all that is needed to induce settlement.

There was no significant difference in degree of settlement on Phyllochaetopterus tubes and on rocks when presented in separate dishes, indicating that both substrata are suitable. When larvae were given a choice of rock vs Phyllochaetopterus tubes, there was a significant difference ($p<0.005$) in degree of settlement indicating a preference for tubes (Table 6). This preference could be due to the greater surface area of the tubes present, as larvae were able to settle on the inner as well as on the outer surfaces. There was no significant difference in degree of settlement on rocks when they were presented alone or

Table 6 - Substratum Preference In Larval Settlement for Solaster spp.

Expt. #	# Larvae per condition	% (#) of Larvae Settled			
		Condition #1 Worm tubes	Condition #2 Worm tubes/Rocks	Condition #3 Rocks	Condition #4 Control
S. stimpsoni					
1	110	24	31	13	31	0
2	30	80	77	3	17	0
3	30	70	33	10	33	3
S. dawsoni					
1	60	63	21	29	54	0
2	90	53	31	26	47	0
3	50	69	43	27	54	0
4	60	88	70	16	73	0
5	40	74	54	5	54	0
S. endeca					
1	30	63	57	7	17	3
2	30	57	57	20	20	7
3	20	25	30	15	25	0
4	20	28	26	21	25	0
S. endeca (F) x S. stimpsoni (M)					
1	30	30	50	17	43	3
2	30	31	37	33	54	0
3	10	30	20	40	40	0

with the worm tubes. The same trend held true for worm tubes. There was no significant difference in settlement on worm tubes when presented alone or with rocks. All three species and S. endeca (female) x S. stimpsoni (male) hybrids showed the same trend when all four conditions were compared. There was a significant difference ($p<0.005$) between species when settlement on rocks in condition 2 and 3 were compared and when settlement on worm tubes and rocks in condition 2 were compared ($p<0.005$) (Table 6). There was considerable variation in the degree of settlement within experiments, which further indicates that the larvae exhibit no substratum preference. The variation between experiments may be due to difference in larval ages. Although the larvae appeared ready to settle that may not have been the case. No preference for shaded undersides of surfaces was observed.

Juvenile Development

Newly metamorphosed juveniles were observed crawling with their mouthes open over rocks, polychaete worm tubes and glass dishes covered with a primary film. If this behavior indicates feeding, the gut is functional soon after the mouth and anus are formed (40-50 d) (Table 5). After 14 months and no supplemental food, 8% (5/64) of S. stimpsoni, 16% (9/55) of S. dawsoni, and 33% (23/70) of S. endeca were still alive. A third set of tube feet had developed in 20% of S. stimpsoni (1/5), 11% of S. dawsoni (1/9) and 13 % of S. endeca (3/23). After 14 months, juveniles of all species had only increased 0.1-0.2 mm in size, to a diameter of 1.55-1.65 mm (Table 4).

Juvenile Distribution

Solaster stars were found in two types of habitats: rocky substratum in fairly shallow water (6-20 m) and among Phyllochaetopterus tubes in deeper water (26-30 m). For individuals of S. stimpsoni, the size (R) range on rocks is 5-282 mm and in the Phyllochaetopterus tubes, 6-121 mm (Table 7). A similar trend is observed for S. dawsoni:

Table 7 - Size Range of S. stimpsoni in Various Locations near Bamfield, B.C.

Location	N	Mean Size (R in mm)	Size Range (R in mm)	R<50 mm (%)	R<25 mm (%)
Rocky Substrata (6-20 m)					
Taylor I.	26	160	6-235	12	4
Aguilar Pt.	66	193	30-250	3	0
Bird Rk.	32	182	11-272	9	3
Edward King I.	34	185	33-265	6	0
Helby I.	11	211	182-282	0	0
Ohiat I.	15	157	54-280	0	0
Scott's Bay	18	230	188-280	0	0
Voss Pt.	30	207	152-255	0	0
Grappler Pt.	36	184	5-245	28	3
Sea Cliff Pt.	21	186	14-247	5	5
Fleming I.	14	150	21-265	14	7
Sea Pool Rks.	28	138	32-213	7	0
Bordelais I.	68	134	34-270	7	0
Phyllochaetopterus Tube Beds (26-30 m)					
San Jose I.	11	14	6-21	100	100
Bamfield Inlet	13	78	28-113	15	0
Wizard I.	3	83	8-121	33	33

a size range from 4-175 mm on the rocky substratum and from 4-95 mm on the Phyllochaetopterus tubes (Table 8). Small asteroids are found in both habitats, but newly metamorphosed individuals (1-4 mm) were never observed. Samples of the tubes were collected frequently and examined in the laboratory under a dissecting microscope to ensure that small individuals were not missed. The fact that other small asteroids were found, eg. Henricia of 2 mm diameter, strongly suggests that small Solaster stars were not being overlooked.

The percentage of small sea stars, R<50 mm, was high on the Phyllochaetopterus tubes (Tables 7,8). Large sea stars were clearly absent from Phyllochaetopterus tube beds. If one compares the density of individuals (Ch. 3, Table 10), little difference was observed between the two habitats. Although the percentage of small Solaster (R<50 mm) are higher in Phyllochaetopterus tubes, smaller specimens (R<25 mm) were also found on rocky substrata at Taylor Island. Juveniles did not appear to recruit to one habitat over the other.

Juveniles were found in the field with their stomach everted on both Phyllochaetopterus tubes and rock surfaces. Stomach contents consisted of detritus, ossicles, shell and small nudibranches were found in two specimens. In the laboratory I tried feeding small Solaster spp. (R<25 mm) small asteroids, small and cut up holothurians. No feeding was observed. I also tried to feed newly metamorphosed S. dawsoni newly metamorphosed S. stimpsoni, but no predation was observed.

Table 8 - Size Range of S. dawsoni in Various Locations near Bamfield, B.C.

Location	N	Mean Size (R in mm)	Size Range (R in mm)	R<50mm (%)	R<25mm (%)
Rocky Substrata (6-20 m)					
Taylor I.	40	50	4-170	70	60
Aguilar Pt.	14	65	12-168	57	29
Bird Rk.	7	77	10-160	29	29
Edward King I.	14	58	6-175	50	36
Helby I.	6	116	27-165	17	0
Ohiat I.	7	92	40-140	29	0
Scott's Bay	2	155	146-164	0	0
Voss Pt.	4	137	116-169	0	0
Grappler Pt.	6	108	8-165	17	17
Sea Cliff Pt.	2	78	29-127	50	0
Fleming I.	3	38	12-68	66	33
Sea Pool Rks.	5	109	67-139	0	0
Bordelais I.	9	90	40-132	22	0
Phyllochaetopterus Tube Beds (26-30 m)					
San Jose I.	4	30	4-95	75	75
Bamfield Inlet	10	21	6-50	90	70

DISCUSSION

Embryonic and Larval Development

The large egg size of all three species of Solaster is typical of the lecithotrophic mode of development. The egg sizes that I have recorded are slightly smaller than those reported for Solaster species in the Northeast Atlantic. Gemmill (1912) found eggs of S. endeca in Scotland to measure 1.000 x 0.800 mm and Falk Petersen (1982) found Solaster sp. eggs of 1.2 mm in diameter in April and May in plankton hauls in northern Norway.

Culturing of lecithotrophic larvae is thought to be straightforward as they do not need to be fed, but embryos of Solaster seem to be very sensitive when they reach the blastula stage, a sensitivity observed by others when rearing lecithotrophic asteroid embryos (M. Komatsu and L. McEdward, pers. comm.). Degeneration and death at this stage may be due to the injection of 1-MA. Such induced spawning may result in the release of eggs that are not fully mature or it may affect egg quality. The position of embryos at the water surface in culture dishes may subject them to different physical conditions (increased exposure to air, light, higher temperatures) which may directly affect the health of the embryos or make them more susceptible to bacterial infection.

The embryonic and larval development of S. stimpsoni, S. dawsoni and S. endeca from fertilization through to metamorphosis was described in detail (Tables 1,2,3,4,5, Fig. 4) and found to be very similar in all three species. The embryonic and larval development described corresponds closely to that reported for S. endeca in the Atlantic Ocean (Gemmill, 1912) and for C. papposus (Gemmill, 1920). Variation between the developmental times of S. endeca, C. papposus and the three Solaster species could be attributed to differences in culture temperature, which was not reported by Gemmill (1912, 1920). Cleavage followed a pattern typical for other echinoderms. The presence of some unequal cleavage in Solaster embryos may not represent abnormal development as similar cleavage pattern was noticed by Gemmill (1912) in a few embryos of S. endeca and is

reported to be common in those of Cribrella oculata (=Henricia sanguinolenta) (Masterman, 1902).

Variation in rate of development within and between cultures of the same species subject to the same conditions, has been observed for other asteroids (Masterman, 1902; Yamaguchi, 1973; Barker, 1978b; Greer, 1962; Gemmill, 1914). Variation between cultures may be explained by differences in temperature, or in other conditions. Individual variation within a culture is not as easily explained. Barker (1978a) suggested that lecithotrophic species, with their yolk reserves, may have a much more synchronous development. There is variation in organic content of S. stimpsoni eggs from different females and among eggs from a single female (McEdward and Carson, 1987). The variation in organic content of eggs may possibly affect the rate of development between and within cultures. Variation in fertilization times may also affect the rate of development within a culture.

The wrinkled blastula stage, observed in all three species of Solaster, is found in 63% (15/24) of asteroid species having lecithotrophic development (Chia, 1966; Hayashi, 1972; Kano and Komatsu, 1978; Komatsu, 1975b; Komatsu, 1982; Komatsu and Nojima, 1985; Komatsu et al, 1979; Lawson-Kerr and Anderson, 1978). This blastula morphology is not a consequence of the large yolky eggs as it is found in eggs of a few planktotrophic species (Hayashi, 1972; Komatsu et al, 1982). According to Hayashi (1972), no relationship can be recognized between the systematic position of a species and the occurrence of a wrinkled blastula, although almost all lecithotrophic sea stars in the Order Spinulosida, for which development has been reported, excluding the Family Echinasteridae, go through a wrinkled blastula stage.

Brachiolarian larvae of the genus Solaster are similar in morphology to those of other lecithotrophic asteroids: Asterina batheri (Kano and Komatsu, 1978), Certonardoa semiregularis (Hayashi and Komatsu, 1971), Mediaster aequalis (Birkeland et al., 1971), Gomophia egyptiaca (Yamaguchi, 1974), and Crossaster papposus (Gemmill, 1920).

Solaster larvae pass through only a brachiolarian stage; a bipinnaria stage is entirely lacking. This pattern is typical for asteroids with large yolky eggs. The larval stage is referred to as a modified brachiolaria as it differs in morphology from the brachiolaria of planktotrophic species.

Uniform ciliation does not facilitate fast swimming (Chia et al, 1984), as the transverse bands of cilia found on nonfeeding larvae of crinoids and holothurians are described to have. The absence of ciliation pattern, and presence of swimming larvae at the bottom of the culture dish, suggests that Solaster larvae are not capable of extensive swimming movements. The tendency of larvae to swim with the posterior end first is the opposite of that described by Gemmill (1912) for larve of S. endeca, but corresponds to movement of the lecithotrophic Indo Pacific coral reef sea star, Gomophia egyptiaca (Yamaguchi, 1974), Ophidiaster granifer (Yamaguchi and Lucas, 1984) and Formia ghardaqana (Mortensen, 1938).

Early attachment of all three species of Solaster is by a sucker, as described by Gemmill (1912) for S. endeca, as larvae were fairly easily detached from the substratum initially, without apparent damage to the sucker. In later stages, this attachment seemed stronger, possibly due to the secretion of an adhesive material, such as the cement described for C. papposus (Gemmill, 1920). At this time, detachment became more difficult and the sucker was often damaged when removed from the substratum. The tips of the brachiolarian arms of Solaster correspond to Barker's (1978a) description of those of Stichaster australis and Coscinasterias calamaria. Adhesive papillae are present but there was no ciliation pattern or secretory droplets evident in Solaster larvae.

Larvae of all three Solaster spp. exhibit a simple torsion involving the folding of the disc towards the oral surface. This torsion is characteristic of larvae of Asterina gibbosa (MacBride, 1896), Henricia sanguinolenta (Masterman, 1902), Crossaster papposus (Gemmill, 1920) and Echinaster echinophorus (Atwood, 1973). The pre-oral lobe of the three species of Solaster it is absorbed into the edge of the disc as in C.

papposus (Gemmill, 1920). This differs from Gemmill's (1912) other description in which the pre-oral lobe of S. endeca was shown to be absorbed into the central mouth region of the oral surface. A large notch in the aboral surface was observed during the absorption of the pre-oral lobe. Gemmill (1912) referred to it as the "nuchal notch" and attributed it to torsion and to subsequent reduction in the walls and internal cavity of the pre-oral lobe. In Solaster larve, the hydrocoel is quadripartite, each part forming a singe podium. This pattern of tube feet formation is similar to that described for Echinaster echinophorus (Kempf,1966). Gemmill (1912) found the hydrocoel in S. endeca to divide into three parts, a terminal foot and a pair of tube feet, with a second pair forming almost immediately afterwards. This pattern was only observed in a few (<5%) individuals of S. stimpsoni. In most cases, two pairs of tube feet form together. The schedule of metamorphic events recorded by Gemmill (1912) for S. endeca corresponds to my findings for all three species of Solaster in the northeast Pacific (Table 5).

Egg colour was maintained throughout development, although newly metamorphosed juveniles were much paler. Colour can be used to identify juveniles of S. endeca, but the juveniles of S. stimpsoni and S. dawsoni are virtually of the same colour and thus difficult to distinguish. When a juvenile of Solaster had completed metamorphosis it usually had its full complement of rays. This is also the case with other, multiple rayed, lecithotrophic asteroids: C. papposus (Gemmill, 1920) and L. hexactis (Chia, 1968). This is in contrast to the multiple rayed planktotrophic sea star of the northeast Pacific Ocean, Pycnopodia helianthoides (Greer, 1962) and the coral reef asteroid, Acanthaster planci (Yamaguchi, 1973). Both of these asteroids have only 5 rays when metamorphosis is complete. Due to the overlap in number of rays between the three Solaster species, ray number is not a good characteristic to use to distinguish juveniles of the three species.

In most invertebrates, metamorphosis involves a drastic change in form. In Solaster and other lecithotrophic asteroids, this change in form is gradual so that it is difficult to define the beginning and end of metamorphosis. In juveniles of M. aequalis (Birkeland et

al., 1971) and in most planktotrophic asteroids, metamorphosis is preceded by settlement on the substratum. In Solaster spp. and in other lecithotrophic asteroids, torsion and development of oral structures did not seem to be retarded for more than a week if a suitable substratum was not found; metamorphosis would occur in the water column. Metamorphosis before settlement is normal for some lecithotrophic asteroids, such as Henricia spp. (Strathmann, 1987) and Astropecten latespinosus (Komatsu, 1975a). Laarvae of Fromia ghardaqana (Mortensen, 1938) and Gomophia egyptiaca (Yamaguchi, 1974) will also undergo metamorphosis in the water column if no suitable substratum is available.

Komatsu (1975a) suggested that developmental time may be a function of egg size, but that it is also related to temperature. Metamorphosis in Solaster larvaae begins after a planktonic period of 8-11 days, but is not complete, until 46-49 days of age. Thus, metamorphosis in Solaster takes 35-41 days. The majority of sea stars with lecithotrophic development take more than 30 days to complete metamorphosis after fertilization (Komatsu, 1975) except for Astropecten latespinosus and Echinaster echinophorus (Atwood, 1973). In contrast, metamorphosis of most planktotrophic asteroids occurs very quickly, usually after settlement. Laarvae of Asterina batheri (Kano and Komatsu, 1978), a tropical species, settle 5 days after fertilization, but only after there are two pairs of tube feet and one terminal tentacle per arm. The process of metamorphosis took five days, not long compared to the 31-45 days reported here in Solaster, but it constitutes half the total larval life of A. batheri. As Komatsu and Nojima (1985) noted, the majority of species undergoing direct development have a brachiolarian larvae, lack a larval mouth, and have a surprisingly long larval life before completion of metamorphosis. The other point to note is that the period of time from settlement, and the beginning of metamorphosis, to opening of the juvenile mouth, is a significant portion of the larval period 78% (31/40) in S. stimpsoni, 88% (35/43) in S. dawsoni, and 78% (38/49) in S. endeca. As there is no loss of larval structures, and it is not a transition phase in which the larva stops feeding until the

juvenile is formed, as seen in planktotrophs, it is not surprising that metamorphosis is such a gradual process. The short planktonic period may reduce larval mortality (Emlet et al., 1987).

Juveniles of S. stimpsoni, S. dawsoni and S. endeca can survive for up to 14 months on a diet of diatoms, detritus and whatever else they can extract from sea water. Strathmann (1987) also found Solaster juveniles to survive for 5 months in unfed cultures. These observations suggest that survival of these juveniles may not be dependent on quality or quantity of food available, although growth may be very dependent on these factors.

Orguro et al. (1976) suggested that, in some asteroids, developmental features are related to the systematic position of the species. This observation seems to hold within the Family Solasteridae. Similarity in larval morphology and size of Solaster species seems to reflect their close evolutionary relationship. Identification between species is difficult except for S. endeca due to similar colouration.

Juvenile Distribution

The patchy distribution of Solaster juveniles in nature suggests that larvae exhibit either some substratum preference or differential mortality. It also indicates that larvae settle on both rocks and Phyllochaetopterus tubes in fairly equal proportions. In the laboratory, larvae did show preference for Phyllochaetopterus tubes when given a choice between them and rocks, but the rocks appeared to be equally suitable when presented alone. In nature, the chance of a larva coming in contact with rocks may be higher than the chance of it coming in contact with Phyllochaetopterus tubes, due to differences in relative abundance of these substrata and differences in depth.

Absence of large individuals on the Phyllochaetopterus tubes can be explained in a number of ways. The food source may not be suitable or sufficient to support a population of large individuals. Body size and population biomass of sea stars has been related to differences in levels of nutrients available for a number of species (McClintock et al.,

1988). There are few large holothurians present, and the asteroid population is substantially lower than that found on rocky substrata. The fact that Solaster juveniles show minimal growth after 14 months (0.1-0.2 mm) when kept in the lab on Phyllochaetopterus tubes or on a fouled mesh surface, indicates that size is not an indication of age and that they can survive on relatively small amounts of food (Table 4). Dayton et al. (1974) found that juveniles of an antarctic asteroid, Pelinaster fucas antarcticus, grew little and retained juvenile form if they did not come in contact with the juvenile food source, sponges of Mycale sp. The transfer from immature to mature morphology seems to be associated with adult food source. If Solaster juveniles cannot find an adequate adult diet, their growth or transition from immature to mature may not occur. The population of small asteroids on Phyllochaetopterus tubes could result from an insufficient source of food and may not indicate recent recruitment.

Birkeland et al. (1971) noted seven species of small (R<10 mm) asteroids, including representatives of S. dawsoni and S. stimpsoni, among beds of Phyllochaetopterus tubes in Puget Sound, Washington. They suggested that these worm tube beds could form an asteroid "nursery", an area favorable for post metamorphic survival. Thus, larvae may preferentially settle into such areas and remain there until a certain threshold size is reached or a period of time has passed and then migrate to the adult habitat. Their hypothesis was further supported by results of settlement experiments which indicated that Mediaster larvae preferentially settle on Phyllochaetopterus tubes (Birkeland et al., 1971). The tubes are thought to be an ideal food source for post metamorphic juveniles (Birkeland et al., 1971) but it is not known whether they provide a better supply of detrital matter than rocks or shells. Worm tubes may provide more protection and camouflage for juvenile asteroids, although, when juvenile Solaster were encountered, they were usually found on the surface of the worm tube beds, where they are no more cryptic than they are on bare rock. Predation pressure on Solaster juveniles may be less in "nursery" areas because of the low number of large asteroids. Since adult S. dawsoni may

eat juveniles, it would be necessary for juveniles to reach a certain size before sharing a habitat with larger conspecifics.

My results do not support this "nursery" hypothesis for Solaster species. Although small Solaster are clearly found on Phyllochaetopterus tubes, there is no indication from my study that beds of Phyllochaetopterus tubes are the only place that they recruite to, as they also occur on rocky substrata. Separation between adult and juvenile habitat has been recorded for other asteroid species. Scheibling (1980) found early juveniles of Oreaster reticulatus to be in sea grass beds of Thalassia testudinum, in which they are very cryptic, not among adults in sea grass beds of Halodule wrightii or on sand flats. Barker (1977) found juveniles of Coscinasterias calamaria to settle only on the coralline algae, Mesophyllum insigne, but to move to other habitats after about one year.

Location of juveniles in the field may not indicate substratum preference, but rather differential survival on different substrata. Migration from juvenile to adult habitat may involve movement over a large distance as the "nursery" areas are not immediately adjacent to adult habitats. Clearly, further information regarding the nutritional requirements of and predation pressures on juveniles is required before we can determine if the distribution of juveniles and adults of Solaster spp. are due to larval substratum choice at settlement, to post settling migration or to differential mortality.

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CHAPTER 3

Reproductive Cycles and Possible Barriers to Hybridization in Three Species of Solaster from the Northeast Pacific Ocean.

INTRODUCTION

Solaster stimpsoni, S. dawsoni and S. endeca are sympatric species found in the northeast Pacific. Their food preferences suggest they overlap in niche as well as in distribution. Adults of S. stimpsoni and S. endeca feed on holothurians and those of S. dawsoni, on other asteroids, particularly S. stimpsoni (Mauzey et al, 1968). All three species of Solaster are broadcast spawners and have similar modes of development (Ch. 2; Strathmann, 1987). Most temperate-water invertebrates exhibit relatively synchronous annual reproductive events within a population, with gamete development occurring in fall and winter and spawning of gametes or planktonic larvae in spring (Thorson, 1946; Giese and Kanatani, 1987). Spawning observations indicate that all three species of Solaster release gametes in spring (Strathmann, 1987, Engstrom, 1974) but their reproductive cycles have not been documented. Do all three species of Solaster exhibit the annual reproductive cycle typical of other temperate water asteroids and is there a spatial and temporal overlap in reproductive season among the three Solaster species?

Adults of Solaster species do not exhibit aggregative or mating behavior prior to spawning. Overlap in habitat and reproductive season, free spawning of gametes, and similarity in larval development suggest that hybridization within the genus may be possible. Can cross fertilization occur between species of Solaster? Hybridization in the lab occurs readily in some echinoderms (Strathmann, 1981; Lucas & Jones, 1976) and does occur in nature between some free spawning echinoderms, such as Echinus esculentus and Echinus acutus in Sweden (Hagstrom & Lonning, 1961) and Holothuria floridana and Holothuria mexicana in southern Florida (Engstrom, 1980).

Structural evidence of hybridization between the three species of Solaster appears to be lacking, although the morphology of these sea stars has not been examined in detail. Hybridization may be difficult to detect, especially if hybrids resembled one parent or the other. Can a Solaster hybrid juvenile be easily distinguished from those of either parent or species? Other studies have described separation in time and/or place of spawning as mechanisms that prevent hybridization (Kingston, 1974; Lucas and Jones, 1976; Falk-Petersen and Lonning, 1983; Lawson-Kerr and Anderson, 1978), or species specificity of binding of sperm to egg during fertilization (Summers and Hylander,1976; Glabe and Vacquier, 1977; Aketa and Ohta, 1979). What barriers to genetic exchange exist among the three species of Solaster and what is the effectiveness of these barriers in maintaining reproductive isolation? The purpose of this chapter is an attempt to answer some of these questions.

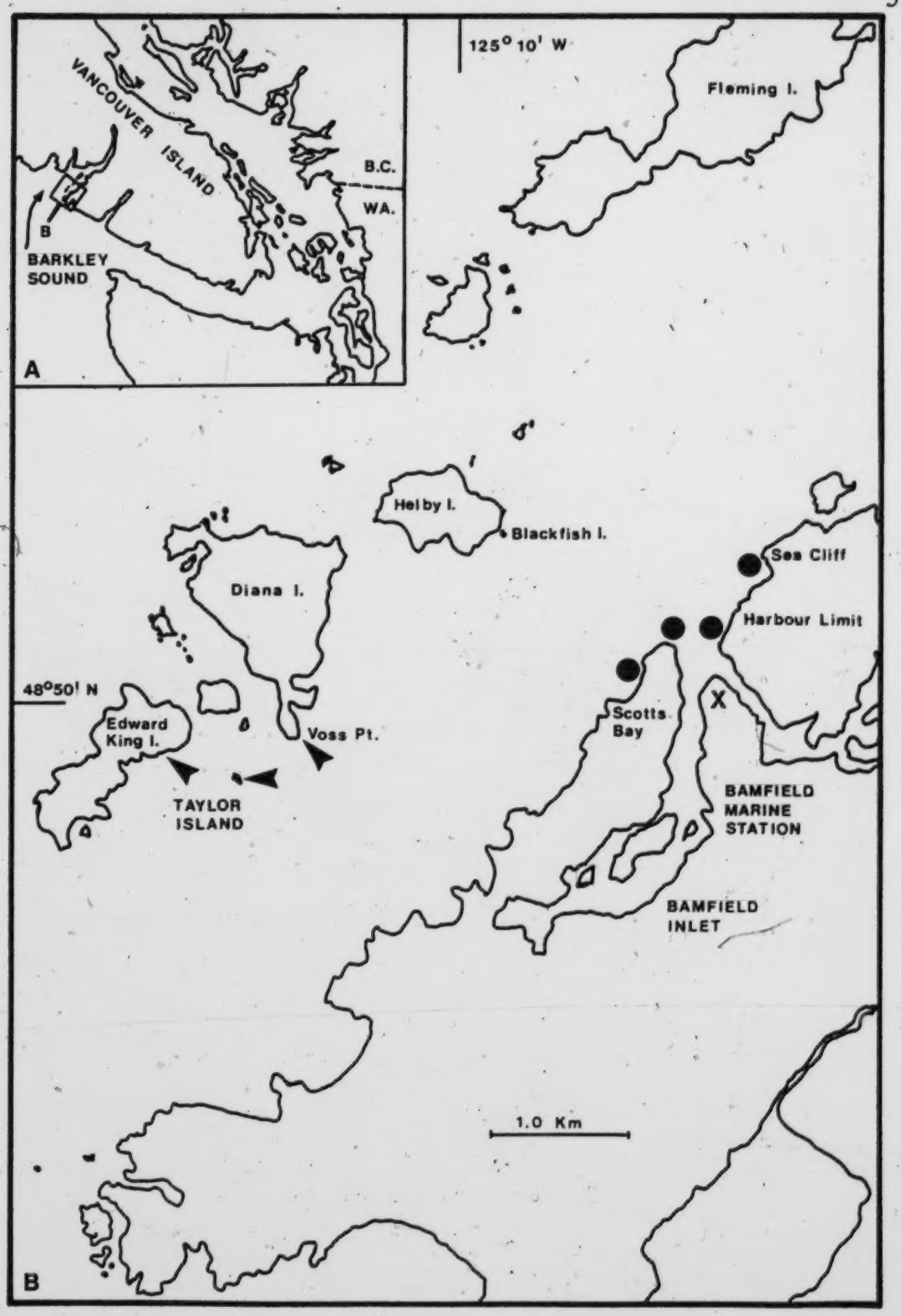
MATERIALS AND METHODS

Gonad Index

Solaster stimpsoni were sampled at approximately monthly intervals from January, 1985 to April, 1987 from Barkley Sound, located on the west coast of Vancouver Island, British Columbia, Canada (48°49'N, 125°08'W) (Fig. 5). Sampling sites were confined to two main areas, near Taylor Island and Bamfield Inlet, which are similar with regard to substratum (rock) and depth (3-20 m). From January, 1985 until January 1986, approximately 20 specimens were collected each month, using SCUBA, from one of the two areas. From February 1986 to April 1987, approximately 10 specimens were collected from each location to insure that site differences were not the cause of variations in data. Each sea star encountered was collected but only specimens having an arm radius (R) greater than 140 mm (>150 g) were included in the analysis. According to Engstrom (1974), S. stimpsoni becomes fertile at a size of roughly 100 g wet weight. Sex could be determined even during the non reproductive months by viewing a gonad smear under a dissecting microscope. The sex ratio in the samples collected was equal except in March, 1985, when only one mature female was found.

Sea stars were held in flow through sea water tables and were dissected within 72 hours of collection. Samples from December, 1985 and January, 1986 were frozen immediately after collection and dissected in February, 1986. Each individual was damp dried, weighed to the nearest 0.01 g, and its arm radius (R) and disc radius (r) were measured. Gonads and pyloric caeca were removed, placed on a paper towel to blot dry for five minutes and then weighed to the nearest 0.01 g. The eviscerated animal was evaluated the same way. Indices of gonads and pyloric caeca were calculated as a percentage of total live body weight (January 1985 to April 1987) and of eviscerated weight (July, 1985 to April 1987) (Giese, 1959). Both indices followed the same trend, although there was a difference in magnitude. The eviscerated gonad index showed more of a peak in spawning in February, 1986, suggesting that variable amounts of water retained in the body cavity

Figure 5 - Location of sites from which Solaster stimpsoni was collected for gonad index data in Barkley Sound, B.C., Canada. Bamfield Inlet Area; circles, Taylor Island Area; arrows.


VANCOUVER ISLAND
B.C.
WA.
B
BARKLEY SOUND
A
125° 10' W
Fleming I.
Helby I.
Blackfish I.
Sea Cliff
Harbour Limit
Diana I.
48° 50' N
Edward King I.
Voss Pt.
TAYLOR ISLAND
Scotts Bay
X
BAMFIELD MARINE STATION
BAMFIELD INLET
1.0 Km
B

reduce the sensitivity of the gonad index when total body weight was used in the calculations. Data using total weights will be discussed as it covers a longer sampling period. Data for both sexes were combined as the organ indices for males and females followed a similar pattern and are of similar magnitude. Arcsine transformations were made on the gonad index data, it was checked for homogeneity of variance and indices at different dates were compared using an ANOVA (Sokal and Rohlf, 1969). Significance was determined at the 5% level. The gonad index was not determined for S. dawsoni or S. endeca as the populations are not large enough to support such sampling.

Oocyte Size Frequency

Gonads of S. stimpsoni were retained from the first 3-5 females dissected, and fixed in Bouins fixative (picric acid, formalin, glacial acidic acid) (Galigher and Kozloff, 1971). When adults of S. dawsoni and S. endeca were encountered (not every month), they were collected too, a small incision in the arm interradius was made and a portion of the gonad was removed and fixed in Bouins fixative (Galigher and Kozloff, 1971). This method did not damage the animal which was returned to the place of collection. Fixed gonad tissues were prepared for histological examination by dehydration in ethanol and embedding in paraffin wax. Series of 10 um sections were cut approximately 400 um apart, and stained with Ehrlich's haematoxylin and eosin (Gurr, 1956).

To determine the size structure of oocyte populations, the mean of two diameter measurements, the greatest diameter and one perpendicular to it, was calculated for 50 oocytes per individual sectioned through the nucleolus. These measurements were grouped into 50 um increments and graphed as a monthly frequency distribution. The small sample sizes for S. dawsoni and S. endeca limit the interpretation of data.

Hybridization Experiments

Induction of spawning and rearing of larvae were conducted by the same methods described in Chapter 2. When a female spawned, ther eggs were randomly subdivided into groups, with one group being fertilized with conspecific sperm. The remaining eggs were fertilized with sperm from a different species of Solaster. Because of great variation in success of conspecific cultures, hybrid cultures were compared to the conspecific cultures from the same batch of eggs. All reciprocal crosses were tried between S. stimpsoni, S. dawsoni and S. endeca. One male S. paxillatus spawned naturally in the lab so was crossed with S. endeca. Unfortunately a ripe female S. paxillatus was never found so the reciprocal cross or a conspecific culture was never established. The rate and pattern of development was compared between the conspecific cultures and their hybrids.

Population Survey

The population of density Solaster sea stars was surveyed by laying a 30 or 50 meter transect line in 4 different locations. The area one meter on either side of the line was surveyed. The number of specimens and arm length (R) were recorded. Substrata within this area were carefully examined, rocks were overturned and crevices searched. Due to the low density of specimens of S. endeca and S. dawsoni, the population was also superficially surveyed by recording the number of individuals of each Solaster species encountered on a dive in 17 locations near Bamfield. The area covered on a dive usually ranged from 500 to 1000 m^2.

RESULTS

Pyloric Caeca Index

There does not seem to be any clear relationship between gonad index and pyloric caeca index (Fig. 6). If transfer of material from pyloric caecum to the gonad occurred, the onset of gonad growth should be preceded or accompanied by a decrease in the pyloric caeca index. The only significant decrease in this index occurred from November, 1985 to March, 1986. In March and April, 1985 and March and April, 1986 when the gonad index showed a decrease due to spawning, the pyloric caeca index increased due to the fact that total weights were used in the index calculations. In the fall of 1985 and 1986 when the gonads increased in size, the pyloric caeca also increased.

Gonad Index

The gonad index shows an annual cycle (Figs. 6,7). This cycle is relatively synchronous throughout the regional population. Organ indices of two local populations, Bamfield Inlet area and the Taylor Island area, were comparable (Fig. 7). The only differences were minor. The Taylor Island population, unlike the Bamfield Inlet population, did not undergo a significant increase from May to June or a significant decrease from June to July in 1986. The index of the Taylor Island population also reached a greater magnitude prior to spawning in 1987 and spawning appeared to start a couple of weeks earlier that year. There were no major differences between the two locations so the data were combined (Fig. 6). The magnitude of the gonad indices are comparable with those reported for other temperate asteroids having lecithotrophic development: Hymenaster membraneceus (Pain et al., 1982), and asteroids with planktotrophic development, Asterias forbesi, A. vulgaris and Pisaster ochraceus (Boolootian, 1966).

Spawning is indicated by a significant decrease in gonad index (Fig. 6). The magnitude of the index prior to spawning does not vary significantly in 1985 (14.84%), 1986 (11.84%) and 1987 (11.28%). The significant decrease ($p<0.05$) in gonad index

Figure 6 - Solaster stimpsoni. Monthly mean gonad and pyloric caeca indices, from Barkley Sound, January 1985 to April 1987. Indices are calculated as a percent of the of organ weight to total body weight. The vertical bars indicate 95% confidence limits and the numbers indicate sample sizes for each datum. Gonad index; squares and solid lines, Pyloric caeca index; circles and dotted lines.

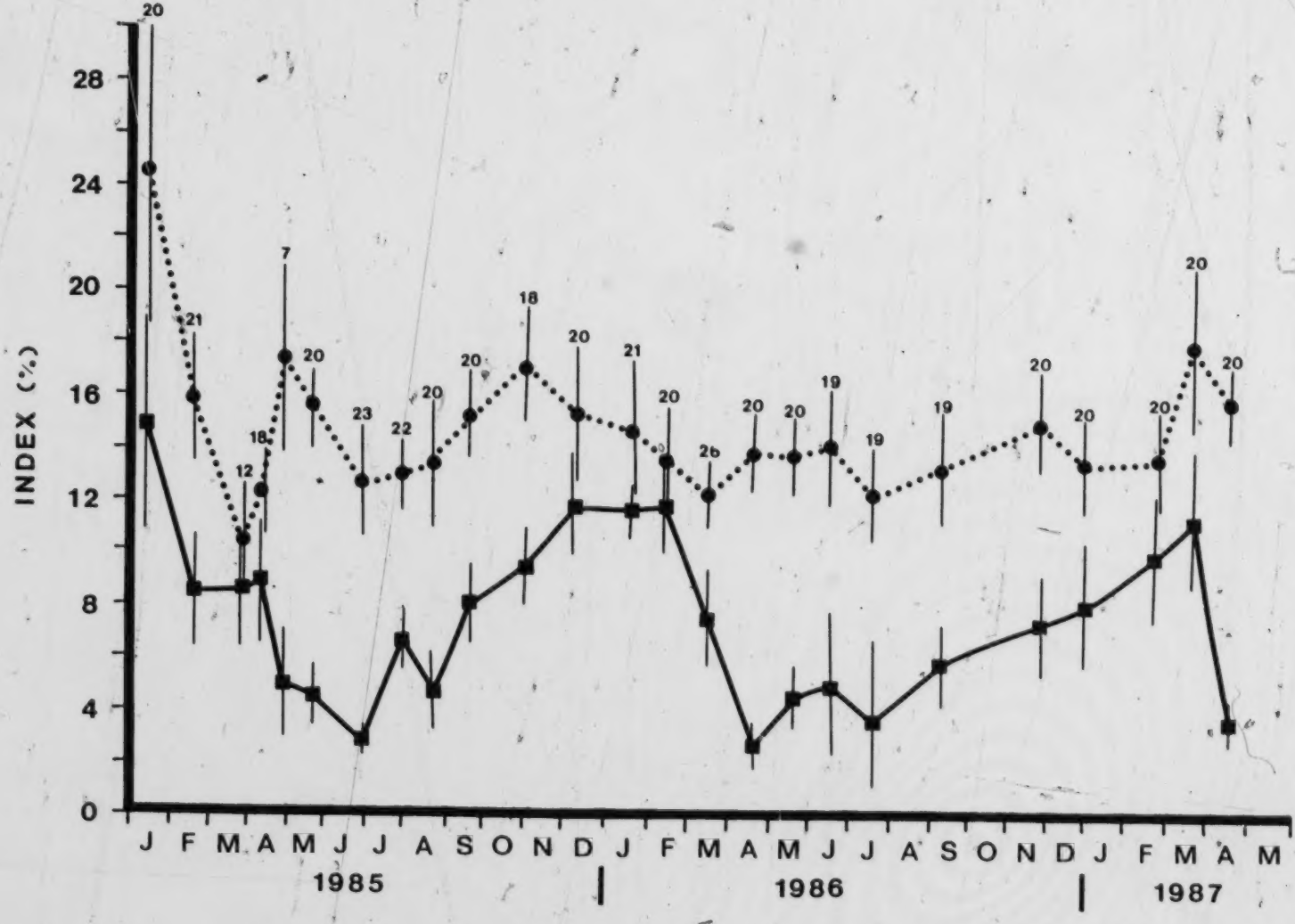
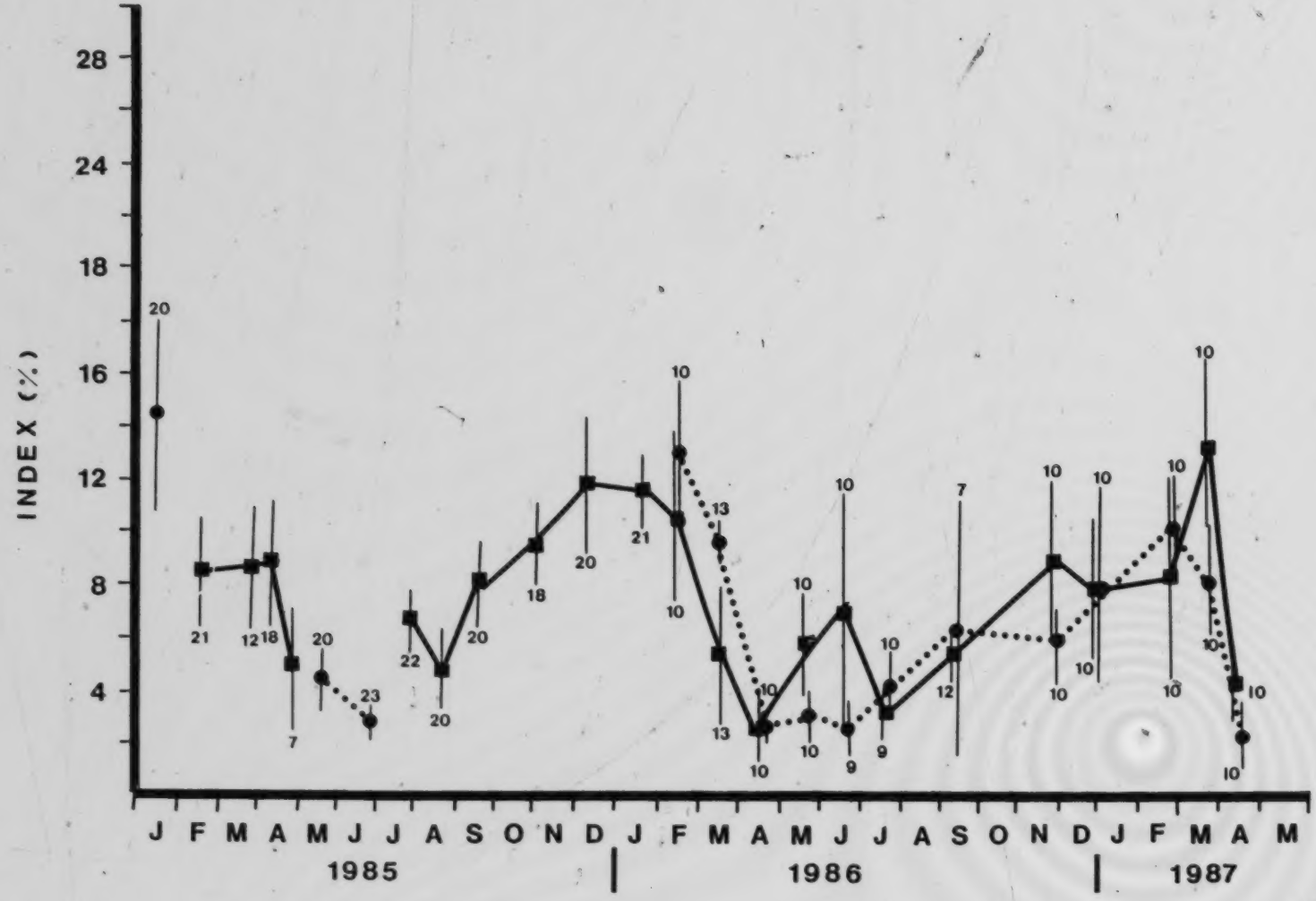

INDEX (%)
0
4
8
12
16
20
24
28
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M
1985
1986
1987

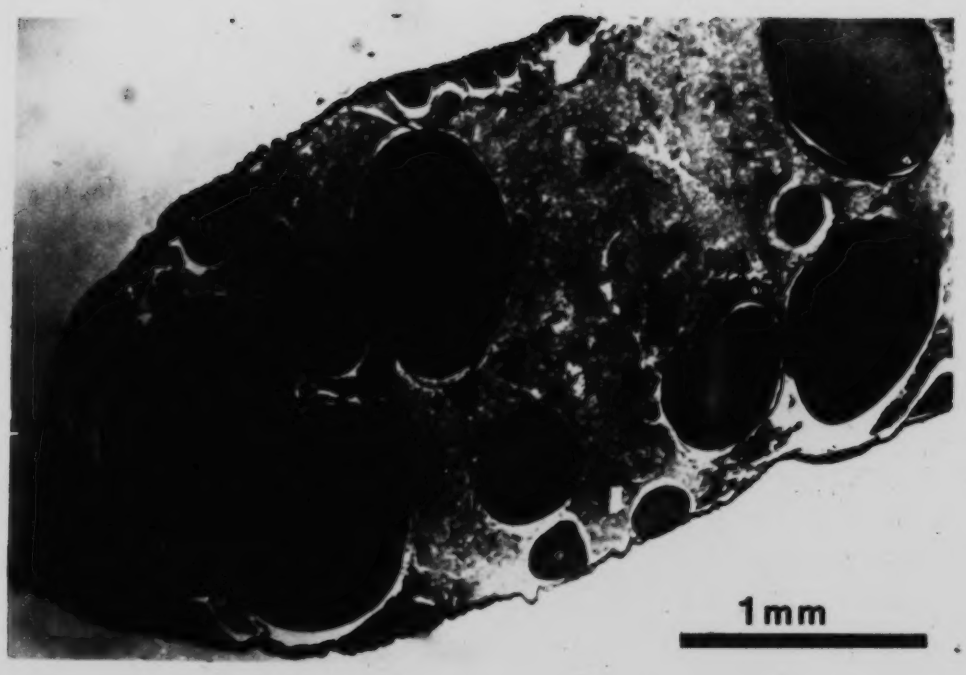
Figure 7 - Solaster stimpsoni. Monthly mean gonad indicies from Bamfield Inlet and Taylor Island Areas, January 1985 to April 1987. Indices are calculated as a percent of gonad weight to total body weight. The vertical lines represent 95% confidence limits and the numbers indicate sample sizes for each datum. Bamfield Inlet Area; squares and solid lines, Taylor Island Area; circles and dotted lines.


INDEX (%)
28
24
18
16
12
8
4
J F M A M J J A S O N D J F M A M J J A S O N D J F M A M
1985
1986
1987

from January to February, 1985, could not be attributed to spawning, because the mean egg diameter (0.5 mm) was less than that found in April (0.9 mm) and fertilization was not possible, suggesting that the eggs were not fully mature in February. A change in body weight due to presence of food in the stomach may affect the gonad index. A comparison of the data with the eviscerated gonad index and feeding observations suggest that this is not a problem. There was little change in gonad index between February and early April. The significant decrease ($p<0.05$) in gonad index from early to late April, 1985, suggests a spawning period. Although the gonad index seemed to still be fairly high in late April, 1-methyladenine (1-MA), which induces spawning during earlier months (Ch. 2; Kanatani et al, 1969), had no effect. When the gonad was opened, a soupy mixture was releasedin more than 75% of specimens examined. The eggs that were still intact were not fertilizable and phagocytosis was occurring (Fig.8). That may explain why the lowest gonad index did not occur until June in 1985 rather than April as seen in 1986 and 1987. This suggests an absence of spawning, rather thana slow release of eggs. 1985 seemed to be an odd year for S. stimpsoni with some individuals possibly spawning in early February, and others not releasing their gametes at all. Some individuals did spawn in April, a pattern which prevailed also during 1986 and 1987.

A significant decrease ($p<0.05$) in the gonad index indicates that spawning occurred over a two month period from February to April in 1986. In 1987, a significant decrease ($p<0.05$) in gonad index occurred from late March to April, indicating a spawning period of about one month. No evidence of an early February spawn or degeneration of oocytes was observed in 1986-87. The gonad index increased very soon after spawning. There was a significant increase ($p<0.05$) from June to July in 1985 followed by a significant decrease ($p<0.05$) in July to August, followed by a significant increase ($p<0.05$) from August to September. In 1986, a similar trend was observed with the gonad index increasing significantly ($p<0.05$) from April to May, decreasing significantly ($p<0.05$) from June to July, and increasing significantly ($p<0.05$) again from July to September. The summer

Figure 8 - Photomicrograph of a cross section through the ovary of Solaster stimpsoni in April 1985, showing degeneration of large oocytes.


1mm

drop could not be attributed to spawning as the animals did not have mature gametes at that time. The gonads appeared to reach full size many months before spawning in 1986. The gonad index illustrates an annual cycle, which is repeated each year but may be altered by environmental effects.

Oocyte Size Frequency

The oogenic cycle of all three species is similar (Figs. 9,10,11). Growing oocytes line the ovarian wall and move into the lumen only when well developed (Fig. 12). A large population of small oocytes (<200 um) appear to be present throughout the year in all three species (Figs. 9,10,11). This suggests that it may take more than one year for an oocyte to fully develop. An abundance of young oocytes is characteristic of a number of shallow water asteroids (Cognetti & Delavault, 1962; Pearse, 1965). Whether these small oocytes undergo normal development and are spawned or are used in further somatic or reproductive growth is not known. The presence of young oocytes throughout the year is consistent with the fact that the gonad index in S. stimpsoni did not drop below 2.79 % (Fig. 6).

There is some seasonal change in the size frequency distribution of oocytes over the course of a year. The change in oocyte size frequency of S. stimpsoni (Fig. 9) during the year corresponds to change in the gonad index (Fig. 6). The percent of large oocytes decreased from February to March but some are present until June. The presence of large oocytes in late spring suggests that not all eggs were not released during the early spring spawning period. Those left over oocytes are probably resorbed. Large oocytes are present again in the late summer and fall. Developing oocytes almost reach their full diameter, many months before spawning (Fig.12).

In S. dawsoni, large oocytes (>500 um) are present until February (Fig. 10). Their disappearance in March would be due to spawning. Oocyte size begins to increase in August and spawning occurs in late February through mid March.

Figure 9 - Solaster stimpsoni. Seasonal changes in the relative size frequency of oocytes during 1985. 50 oocytes per female were measured and 3-5 females were examined each month. The scale line indicates the percent of oocytes at a particular size.

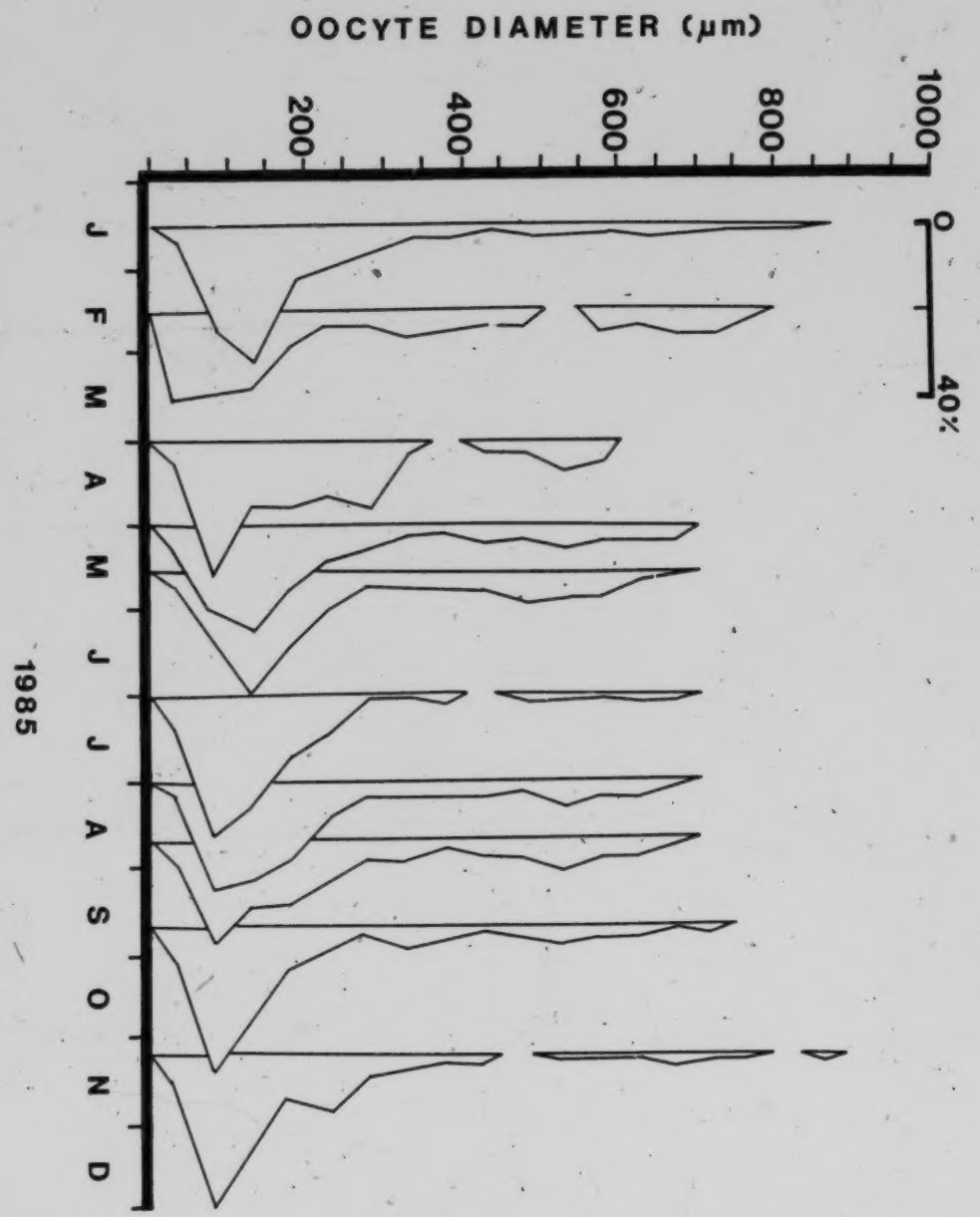

OOCYTE DIAMETER (µm)
200
400
600
800
1000
0
40%
J F M A M J J A S O N D
1985

Figure 10 - Solaster dawsoni. Seasonal changes in the relative size frequency of oocytes during 1985. 50 oocytes per female were measured and 1-4 females were examined for each sampling date. The scale line indicates the percent of oocytes at a particular size

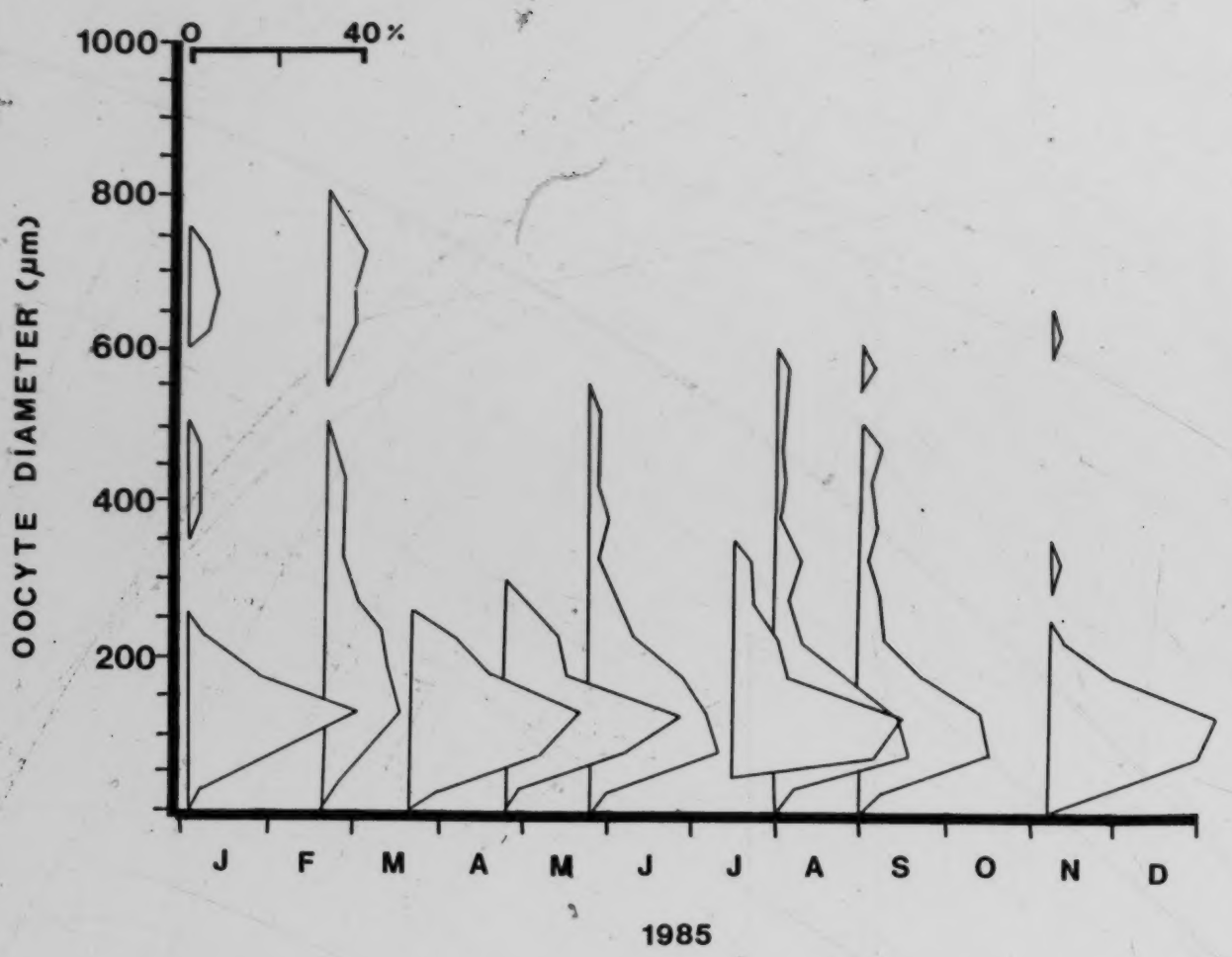

0
40%
1000
800
600
400
200
OOCYTE DIAMETER (µm)
J
F
M
A
M
J
J
A
S
O
N
D
1985

Figure 11 - Solaster endeca. Seasonal changes in the relative size frequency of oocytes during 1985. 50 oocytes per female were measured and 1-3 females were examined for each sampling date. The scale line indicates the percent of larvae at a particular size.

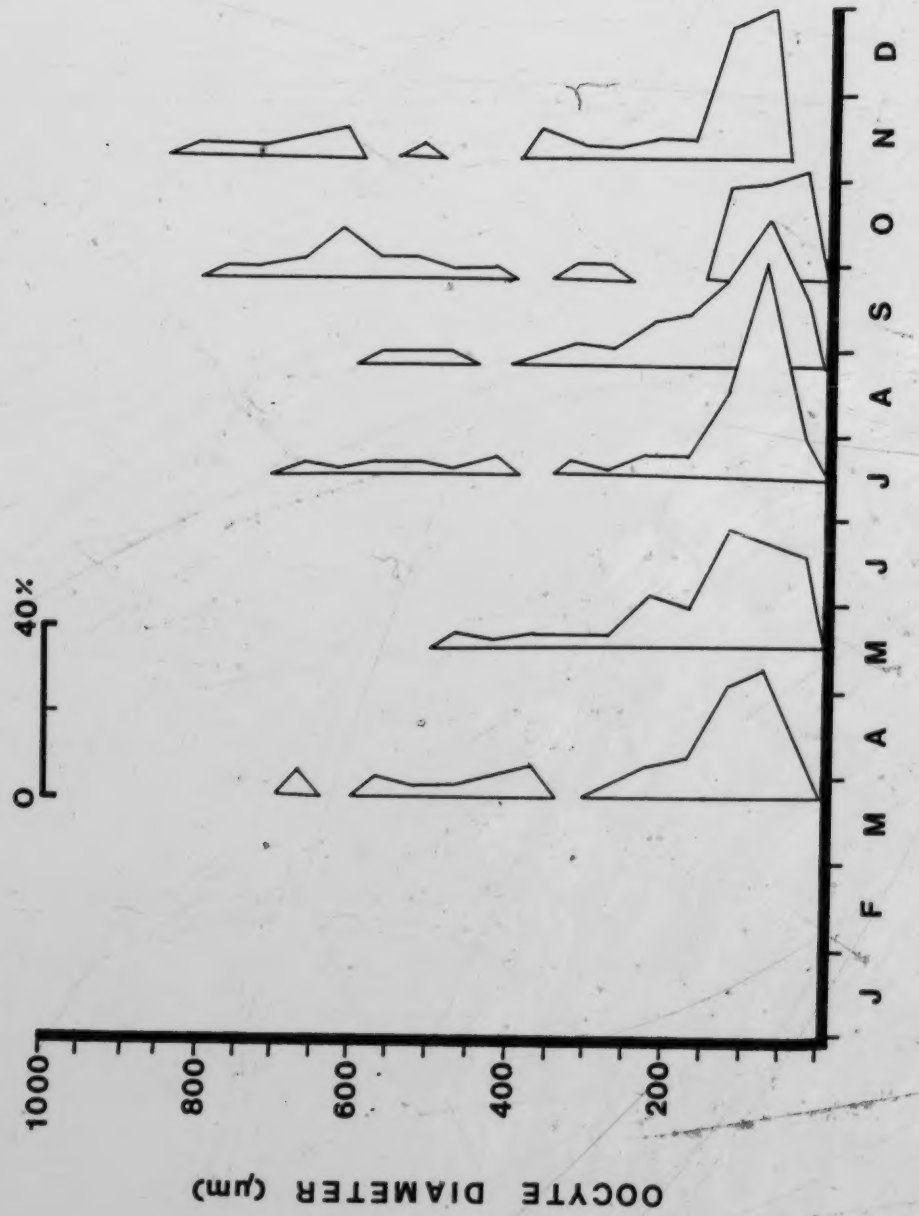

OOCYTE DIAMETER (µm)
1000
800
600
400
200
J F M A M J J A S O N D
1985
0
40%

Figure 12 - Photomicrographs of ovary cross sections of Solster stimpsoni collected November 1985.

1mm

The reproductive cycle is not as clear for S. endeca, although that may be a result of the small sample size (Fig. 11). A predominance of small oocytes is observed throughout the year. The number of large oocytes decreased from March to May, suggesting it to be the time of spawning. Large oocytes appeared again in September, indicating gonadal growth.

Spawning Observations

Spawning individuals of Solaster species assume the characteristic spawning posture for asteroids, rising up on their arm tips and lifting the disc off the substratum. Observations of natural spawning are the best indication of when spawning occurs in the field. Spawning of S. stimpsoni and S. dawsoni has been observed in April and May in Barkley Sound (Table 9). The fact that both S. dawsoni and S. stimpsoni were seen spawning on the same day at the same time about 10 m from each other indicates that both species may be responding to the same spawning cue or possibly triggering each other. In the laboratory, spawning of S. stimpsoni could be induced with 1-MA (10^{-4} M) from late February to mid-April, S. dawsoni could be induced from mid-March to mid-April and S. endeca could be induced from mid-February to mid-April.

Observations of both natural and 1-MA induced spawns and those made during gonad index dissections, suggests ta single spawning period for each individual rather than a series of spawning events in which only a portion of the gametes are released. Two individuals were found in which the gonads on one side of the body were empty and those on the other half were still full. Usually, all gonads within an individual were in the same state of fullness.

Distribution

All three species of Solaster occupy the same habitat (Tables 10,11). S. endeca is uncommon, but, when found, usually occurs with S. stimpsoni and/or S. dawsoni. Dredging and submersible observations indicate that they are not found in greater

Table 9 - Dates for Natural Spawning Observations for S. stimpsoni and S. dawsoni

Species	Date	Location	Sex	Notes
S. stimpsoni	April 9/85	Scott's Bay, Bamfield	males, females	spawned during collection and transport to laboratory
S. stimpsoni	April 28/85	Scott's Bay, Bamfield	males	spawned during collection and transport to laboratory
S. stimpsoni	May 19/85	BMS Lab, Bamfield	male	natural spawn observed in the laboratory
S. stimpsoni	April 1/87	Ohiat I., Bamfield	male	observed by J. Elliot
S. stimpsoni	April 2/87	Blackfish I., Bamfield	male	observed by J. Elliot next to spawning S. dawsoni
S. dawsoni	April 2/87	Blackfish I., Bamfield	male	observed by J. Elliot next to spawning S. stimpsoni

Table 10 - Distribution of S. stimpsoni, S. dawsoni and S. endeca near Bamfield, B.C. Transect Survey

Date	Location	Area (m^2)	# Observed (Density [#/m^2])		
			S.stimpsoni	S.dawsoni	S.endeca
Rocky Substrata (6-20 m)					
May 16-24/85	Taylor Island	300	9 (0.03)	15 (0.05)	0
August 29-31/85	Taylor Island	200	6 (0.03)	11 (0.06)	0
June 28/85	Edward King I.	60	8 (0.13)	0	0
August 6/85	Helby Island	60	7 (0.12)	2 (0.03)	0
Phyllochaetopterus Tube Beds (26-30 m)					
August 20-23/85	Bamfield Inlet	100	4 (0.04)	6 (0.06)	1 (0.01)

Table 11 - Distribution of S. stimpsoni, S. dawsoni and S. endeca near Bamfield, B.C. - Diving Observations

Date	Location	No. Observed		
		S.stimpsoni	S.dawsoni	S.endeca
January 12/85	Voss Point	24	2	0
February 19/85	Grappler Point	20	7	1
March 26/85	Harbour Limits	12	1	1
April 9/85	Scott's Bay	19	2	0
April 25/85	Grappler Point	25	9	0
May 6/85	Sea Pool Rocks	3	2	0
June 9/85	Edward King Island	8	9	0
June 28/85	Edward King Island	28	7	1
July 12/85	Ohiat Island	14	5	3
July 27/85	Aguilar Point	27	9	0
August 2/85	Bordelais Island	34	3	1
August 6/85	Aguilar Point	11	2	0
August 13/85	Bordelais Island	34	6	2
August 24/85	Sea Pool Rocks	25	3	2
February 5/86	Ohiat Island	3	0	1
February 8/86	Harbour Limits	3	1	0
February 10/86	Fleming Island	15	1	0
February 11/86	Ohiat Island	0	2	2
February 13/86	Blackfish Island	16	5	6
March 10/86	Blackfish Island	21	3	0
March 11/86	Sea Cliff Point	13	1	1
March 14/86	Fleming Island	18	0	0
March 17/86	Fleming Island	15	1	0
March 18/86	Harbour Limits	23	0	0
March 22/86	Bordelais	44	1	0
April 3/86	Scott's Bay	11	3	1
April 8/86	Scott's Bay	11	5	0
April 9/86	Blowhole	18	5	0
April 16/86	Execution	9	3	0
April 18/86	Dianna Island	17	8	0
April 12/86	Voss Point	18	10	2
April 24/86	Voss Point	16	5	0
May 23/86	Bamfield Inlet	4	1	1
May 25/86	San Jose Islet	3	2	1
July 2/86	Bamfield Inlet	1	1	0
July 26/86	San Jose Islet	1	2	0
July 27/86	Edward King Island	10	1	0
August 27/86	San Jose Islet	8	1	0

abundance in deeper water. No evidence of spawning aggregations during the breeding season was observed. The density of all three species was fairly low, with S. stimpsoni being most common and reaching 0.13/m^2 and S. endeca least common, at densities of 0.01/m^2 (Table 10). Although S. dawsoni was present in higher density than S. stimpsoni at Taylor Island in May and August (Table 10), it should be noted that 19 of the 24 S. dawsoni encountered had a radius (R) of less than 26 mm, while only 2 out of 16 S. stimpsoni were that size. The density of juveniles appears to be higher for S. dawsoni than for S. stimpsoni, although, when small individuals of the two species are often hard to distinguish. Juveniles may have been overlooked when transects were not laid (Table 11). The difference in survey technique may explain the difference in relative abundance between S. stimpsoni and S. dawsoni in Tables 10 and 11. Engstrom (1974) documented the distribution of these Solaster species in the San Juan Islands and also found them in close proximity to each other. Diving observations of Mauzey et al (1968) in Puget Sound also illustrate that all three species occur in the same habitat.

Hybridization

Some fertilization occurred in all reciprocal crosses between S. stimpsoni, S. dawsoni and S. endeca (Table 12). Fertilization success was recorded as an estimate of the percent of eggs fertilized. The percent fertilization in crosses was often lower than in conspecific cultures (Table 12,13). Excess sperm were added to cultures to ensure that the sperm concentration was not a factor in fertilization success. Degree of hatching also varied considerably between cultures and was lower for S. endeca female and S. dawsoni female crosses than in conspecific cultures (Table 12). The hatching success of most S. stimpsoni hybrids was identical to that of control, conspecific cultures (Table 12).

At least one larva completed metamorphosis in all control cultures, but in only about half of the hybrid cultures. At least one larva metamorphosed in all crosses, except when S. stimpsoni eggs were fertilized with sperm from S. endeca (Table 13). Percent larvae per

Table 12 - Results of Hybridization Experiment Among Solaster spp.

Culture #	Species female	male	% Fertilize	% Hatch	% Metamorphose	Developmental Stage Reached	# alive after 2 m (months)	13 m
1	S.stimpsoni		80	50	<1	juvenile	2	0
1	S.stimpsoni	x S.dawsoni	80	50	0	gastrula		
2	S.stimpsoni	x S.stimpsoni	60	10	6	juvenile	26	1
2	S.stimpsoni	x S.dawsoni	60	10	0	brachiolarian		
3	S.stimpsoni		50	20	<1	juvenile	0	0
3	S.stimpsoni	x S.endeca	10	0	0	blastula		
4	S.stimpsoni		50	20	2	juvenile	5	0
4	S.stimpsoni	x S.dawsoni	60	20	<1	juvenile	0	0
4	S.stimpsoni	x S.endeca	60	20	0	brachiolarian		
5	S.dawsoni		90	20	9	juvenile	106	7
5	S.dawsoni	x S.stimpsoni	80	5	<1	juvenile	0	0
5	S.dawsoni	x S.endeca	60	5	<1	juvenile	5	0
6	S.dawsoni		70	25	14	juvenile	72	4
6	S.dawsoni	x S.stimpsoni	40	0	0	fertilization		
7	S.endeca		80	20	8	juvenile	31	16
7	S.endeca	x S.stimpsoni	60	2	0	gastrula		
7	S.endeca	x S.paxillatus	40	2	<1	juvenile	1	0
8	S.endeca		90	5	1	juvenile	0	0
8	S.endeca	x S.dawsoni	90	10	<1	juvenile	1	0
9	S.endeca		60	25	4	juvenile	19	1
9	S.endeca	x S.stimpsoni	50	20	<1	juvenile	0	0
10	S.endeca		80	25	8	juvenile	38	0
10	S.endeca	x S.stimpsoni	50	5	2	juvenile	0	0
10	S.endeca	x S.dawsoni	5	0	0	fertilization		
11	S.endeca		80	50	7	juvenile	12	5
11	S.endeca	x S.stimpsoni	40	5	1	juvenile	6	3
11	S.endeca	x S.dawsoni	5	0	0	fertilization		
12	S.endeca	x S.stimpsoni	70	10	8	juvenile	0	0

Table 13 - Summary of Results of Hybridization Experiments Among the Genus Solaster

Species Female	Male	# Cultures Started	Fert. (%)	# Cultures to reach each stage Blastula	Gastrula	Brachiolaria	Juvenile (%)
S.stimpsoni	S.stimpsoni	4	4 (50-80)	4	4	4	4 (<1-6)
S.stimpsoni	S.dawsoni	3	3 (60-80)	3	3	2	1 (<1)
S.stimpsoni	S.endeca	2	2 (10-60)	2	1	1	0
S.dawsoni	S.dawsoni	2	2 (70-90)	2	2	2	2 (9-14)
S.dawsoni	S.stimpsoni	2	2 (40-80)	1	1	1	1 (<1)
S.dawsoni	S.endeca	1	1 (60)	1	1	1	1 (<1)
S.endeca	S.endeca	5	5 (60-90)	5	5	5	5 (1-8)
S.endeca	S.stimpsoni	5	5 (40-70)	5	5	4	4 (<1-8)
S.endeca	S.paxillatus	1	1 (40)	1	1	1	1 (<1)
S.endeca	S.dawsoni	3	3 (5-90)	1	1	1	1 (<1)

culture which metamorphosed was variable but was always lower for hybrid cultures than for conspecific cultures. Less than 1% metamorphosised in all hybrid cultures except S. endeca eggs fertilized with sperm from S. stimpsoni (Tables 12,13). It is difficult to interpret the success of hybrids because there was so much variation in culture success of conspecific cultures. Success of a culture may depend upon culture conditions (temperature, salinity, oxygenation), sperm concentration and egg condition (Ch. 2). The number of hybrid cultures started was considerably less than those of conspecifics, so differences in culture success could also be a function of sample size.

Embryonic and larval development of hybrids was similar to that of conspecifics (Ch. 2) with regards to morphology, chronology of developmental events (Table 14) and size (Table 15). Hybrid larvae of a particular cross were almost impossible to distinguish from other hybrids or conspecifics. Arm number, which is evident before completion of metamorphosis (Ch. 2), varied between arm number reported for each parent species. Hybrids in which S. endeca was the mother, retained the bright orange colour of the eggs. Hybrids in which S. stimpsoni or S. dawsoni were the mother were a pale greenish-brown.

Solaster juveniles which are 3 and 14 months of age were similar in size. Although about half of the control cultures had living juveniles after 14 months, the only hybrids that were alive 3 months after metamorphosis were progeny of: S. dawsoni x S. stimpsoni, S. stimpsoni x S. paxillatus, S. stimpsoni x S. dawsoni and S. endeca x S. stimpsoni. After 14 months, the only hybrid still alive was S. endeca x S. stimpsoni. No hybrids or conspecifics were reared through to reproductive maturity, as this is thought to take 4-4 1/2 years (Engstrom, 1974).

Table 14- Chronology of development of Solaster hybrids at 10-12 °C

Stage of development	F M	S. stimpsoni S.dawsoni	S.endeca	S. endeca S.dawsoni	S.stimpsoni	S. dawsoni S.stimpsoni	S.endeca
4 cell				4.5 h	7.5 h		
wrinkled blastula		33 h	34.5 h	35 h	35.5 h	31 h	
hatching		53 h		74 h	62.5 h	53.5 h	
brachiolarian				13 d	19 d	19.5 d	
attachment		24 d	18.5 d	24 d	25 d	20 d	
5 hydrocoel pouches		24 d				20 d	
8 sets tube feet		39 d		52.5 d	41 d		
10 sets tube feet		39 d					
loss of attachment						47 d	
mouth open					57 d		

* Developmental times are expressed as an average of the time it took the majority of individuals in a culture to reach that stage.

Table 15 - Size of Larvae and Juveniles of Solaster Hybrids

Stage of Development	Size (X Length x X Width mm)						
Female	S.stimpsoni		S.dawsoni		S.endeca		
Male	S.endeca	S.dawsoni	S.stimpsoni	S.endeca	S.stimpsoni	S.dawsoni	S.paxillatus
early brachiolarian					1.1 x 0.7		
brachiolarian					1.6 x 1.1		
5 hydrocoel pouches			1.0 x 0.9				
juvenile, 6 set tube ft.			1.2 x 1.1	1.2 x 1.1	1.4 x 1.3		
juvenile, 9-12 set tube ft.		1.4 x 1.1	1.4 x 1.3	1.3 x 1.0	1.4 x 1.3	1.3 x 1.2	
juvenile, mouth open			1.6 x 1.5	1.4 x 1.3	1.5 x 1.5	1.3 x 1.2	1.7 x 1.7
14 month old juvenile					2.0 x 2.0		

DISCUSSION

Reproductive Cycle

All three Solaster species exhibit a distinct, annual pattern in their reproductive cycles, with gonad development occurring throughout the year and spawning occurring in spring. The presence of small oocytes throughout the year suggests that oocytes take more than one year to develop, a pattern which has been observed in other temperate asteroids having lecithotrophic larvae (Chia, 1968). The rate of gametogenesis may be affected by food abundance, exposure, currents, temperature, salinity and chemical factors (Giese and Pearse, 1974). As all three Solaster species have lecithotrophic larvae, it is not necessary that the larval stage coincide with the time when planktonic food is available. It may be important though for juveniles to recruit in the summer when water temperature is high and there is an abundance of food in the form of newly settled organisms. Or it may be a mechanism to reduce predation on the embryos and larvae as there is a lot of plankton available for filter feeders to consume in the spring.

The translocation of stored material (glycogen, lipid, and protein) from the pyloric caecum to the gonad, which is clearly seen in other asteroids (Mauzey, 1966; Chia, 1966; Nimitz, 1971; Menge, 1975; Jangoux and van Impe, 1977), is not obvious from the data obtained for S. stimpsoni. Farmanfarmaian et al. (1958) suggested that these digestive glands are used to stockpile nutrients during months when feeding is most intense. Later, these storage products are transferred to the maturing gonads. Females of Pisaster giganteus do not show seasonal fluctuations in size of pyloric caeca when food supply is good, but, it has been shown that somatic maintainance and growth take priority over gonad development when food is limited (Harrold and Pearse, 1980). Observations of feeding made while diving and the presence of food in the stomach during examination for the gonad index indicates that Solaster females appear to feed year round in the Barkley Sound area. Neither did Engstrom (1974) find evidence of seasonal feeding periodicity in S. stimpsoni in the San Juan Archipelago. It thus seems unlikely that variations in pyloric

caecal index (Fig. 6) are due to variation in food availability . Solaster species thus appear to be fairly atypical among asteroids in not using the pyloric caeca as a storage organ. The advantage of stock piling nutrients in the pyloric caeca rather than forming a gonad immediately, is not clear.

Gonad index data, histological study of ovaries and natural spawning observations reveal similar reproductive patterns to exist in all three Solaster species. The spawning season spans a period of one to two months and a long spawning period suggests a lack of synchrony among individuals. Observations that males of S. dawsoni and S. stimpsoni spawned at the same time of day in the same place (Table 9) suggest that they are responding to the same spawning cue. Environmental stimuli such as day length, temperature change, phases of the moon, tidal rhythms, salinity changes, periodic upwellings and plankton blooms have been suggested as spawning cues for other invertebrates (Giese and Kanatani, 1987). As all three Solaster species occur in relatively shallow water, they are subject to the same seasonal fluctuations in the physical-chemical environment. Changes in photoperiod or water temperature, which are thought to trigger spawning in other asteroids (Giese and Kanatani, 1987), could do so as well in Solaster spp. There is no large, seasonal change in water temperature in Barkley Sound (7.5-13 °C at the surface and 8-11 °C at 25 m), so change in photoperiod may be more important. Pearse and Eernisse (1982) found that long day lengths in spring and summer seemed to synchronize initiation of gametogenesis and gonadal growth in fall for Pisaster ochraceus. Pearse and Walker (1986) demonstrated that photoperiodic regulation of reproduction is important in Asterias vulgaris, an asteriod that normally experiences marked seasonal changes in sea temperature. Olive (1984) has suggested that a complex hierarchy of different factors may act synergistically to ensure that reproduction takes place at the most suitable time at a given locality and that natural selection will act to maintain the efficiency of these factors if larval success and ultimately recruitment depend upon it. Variation in

reproductive cycle from year to year suggests that Solaster is reponding to action of some environmental cue or cues.

Information regarding egg and sperm viability is important in evaluating the importance of overlap in spawning time for males and females. Must it be within days, hours or minutes? This information is also important when considering the probability of hybridization between the three species of Solaster. Pennington (1985) demonstrated that, for Strongylocentrotus droebachiensis, eggs remain viable for 90 minutes and sperm remain viable for only 20 minutes. When life of gametes is short, synchronization of spawning and proximity of the sexes is critical to reproductive success. Miller (1985) has shown that although sperm may not remain active for long, certain substances will reactivate them. Eggs and sperm either must be released in close proximity to each other or mixing must bring them into contact. Miller (1985) demonstrated sperm chemotaxis in asteroids, and showed that sperm of all three species of Solaster respond to conspecific and congeneric egg extracts.

Decrease in size of gonads in females of S. stimpsoni during the spring of 1985 was partly due to breakdown or resorption of gametes, rather than to their release. This process suggests that appropriate spawning cues were not received or that there was some physiological malfunction in the females. The eggs may degenerate if not released during the peak spawning period (March to early April). Resorption rather than release may be advantageous if the chance of eggs developing normally is low, so that the nutritive material of the egg is not lost. The absence of spawning cues may reflect poor environmental conditions for larval development. Similar internal oocyte degeneration has been described in certain shallow and deep water asteroids that produce large eggs (Shick et al., 1981; Tyler et al., 1982; Tyler and Pain, 1982).

Hybridization

Development through to metamorphosis occurred in progeny of all reciprocal crosses between the three Solaster species except when S. stimpsoni oocytes were fertilized with S. endeca sperm, although only two cultures were started. Hybridization among asteroids is not common and results of crosses between other asteroids show evidence of gamete incompatibility (Tyler and Tyler, 1966). Gemmill (1912) tried to hybridize S. endeca and Crossaster papposus and he found that the ova and spermatozoa of each species were mutually strongly attracted. Fertilization was possible and normal larvae developed but metamorphosis never occurred. Gemmill (1914) found too that fertilization occurred and development was possible through to blastula or gastrula between reciprocal crosses of Asterias rubens and Marthasterias glacialis. Gemmill (1916) also found cross fertilization and development through to early bipinnaria to be possible between Stichastrella rosea oocytes and M. glacialis sperm and Barker (1978), found that fertilization of Sticaster australis oocytes by Coscinasterias calamaria sperm was possible with development of hybrids through to gastrula. Asteroid hybrids have only been reared successfully in the laboratory in the case of Acanthaster planci x A. brevispinus (Lucas and Jones, 1976). Hybridization between echinoids of quite divergent taxa has been reported for a number of cases (Hinegardner, 1975).

Maintenance of specific identity in sympatric populations of closely related species is only possible when either a prezygotic or postzygotic mechanism exists to ensure effective reproductive isolation. Prezygotic isolating mechanisms should be favoured over post-zygotic mechanisms to prevent waste of reproductive effort (Ayala, 1978). Hybridization between adults of three species of Solaster was possible in the laboratory, but the developmental success of hybrid progeny was reduced. The possibility of hybridization in the field does seem good as there is no evidence of spatial or temporal separation between the three species during spawning. There are no ethological or mechanical barriers to cross-species reproduction since there is no mating behavior and

fertilization is external. Miller (1985) reported that gametic isolation is probably not present as sperm do not discriminate among attractants in a species-specific manner within the family Solasteridae, although they do so in members of many other echinoderm families.

If a premating barrier to hybridization does exist it must consist of very small differences in the time or place of spawning. S. endeca is found in very low densities in Barkley Sound and the San Juan Archipelago (Engstrom, 1974) and the chance of egg and sperm meeting could be very low. Hybridization may be occurring simply because the eggs and sperm may not encounter conspecific gametes. The population in this area may not be reproductive and the larvae may be coming from areas of higher density.

Post-mating barriers may consist of substantially reduced viability, fecundity or fertility of hybrids in nature. Hybridization may result in reduced reproductive efficiency of the three species, due to a lower success rate of cross relative to conspecific fertilizations. Kingston (1974) found that hybridization between the cockles, Cardium edule and C. glaucum was possible but that larvae of reduced vigour were produced. He suggested the reduced success of interspecific crosses to be of particular significance in sympatric populations where it could result in reduced reproductive efficiency in both.

The three Solaster species appear to be maintaining reproductive isolation through action of some postzygotic isolation mechanism as there is no significant separation in time and place of spawning, fertilization is possible and sperm of the different species are mutually attracted to eggs. Further work is necessary to determine the probability of eggs and sperm of different species meeting and their viability. Observations of spawning in nature will provide further evidence as to whether adults of each species are spawning together on a regular basis. The reduced viability of hybrids is probably the main postzygotic mechanism. The fact that these hybrids were not reared to maturity limits the story to a certain extent. Very little can be said about barriers and potential hybrids when information on survivorship and fertility is not available. Further investigation is necessary before we can clearly understand how species of Solaster remain reproductively isolated.

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CHAPTER 4

GENERAL DISCUSSION AND CONCLUSION

The main purpose of this study was to provide some information on the reproductive biology of speces of Solaster, a predominant asteroid genus in the shallow, subtidal of the northeast Pacific Ocean. I also hoped to determine how reproductive isolation is maintained between the three species of Solaster in this area through a comparison of their reproductive biology.

The embryology of S. stimpsoni, S. dawsoni and S. endeca was described and compared from fertilization through metamorphosis. All three species are broadcast spawners which produce lecithotrophic modified brachiolarian larvae very similar in size and morphology. Chronology and pattern of embryonic and larval development was also very similar between species, taking 8-11 d to settlement and 40-50 d to complete metamorphosis at 10-12 ^{o}C.

Further work is needed to understand and circumvent the problems encountered in raising lecithotrophic embryos. Comparison of induced and natural spawning and use of different culturing techniques may provide some answers. Comparison of rates of development of different egg sizes, and of eggs released early or late during the spawn may provide information explaining the variation in rates of development among cultures.

The low percentage of larvae which metamorphosed in the laboratory may not be due to culturing techniques, but what you would expect to find nature due to the low probability of the eggs and sperm meeting and because of predation. The percentage which successfully recruit back to the population is probably very low since newly metamorphosed juveniles were not found in the field. Rates of recruitment of Solaster may affect, in turn, the density of Cucumaria miniata and other sea stars.

Individuals of all three species occupy the same habitat. Juveniles, or small individuals, occur on Phyllochaetopterus worm tubes, and at similar densities on rocky substrata where large individuals also occur. Feeding experiments in the laboratory, and

transfer and cage experiments in the field would be necessary to determine if the small size sea stars on the Phyllochaetopterus tubes is due to diet or to whether they recruit to such habitats and then move to other habitats as they increase in size. Preliminary observations in the laboratory indicate that Solaster juveniles can survive for extended periods with little food and show little growth. My data show that Solaster larvae will settle on rocks or Phyllochaetopterus worm tubes and suggest that survival and growth may depend on type of food available. A closer examination of larval arms and sucker may reveal the degree to which Solaster sea stars can detect the substrata and how attachment is maintained. Tagging of juveniles in the field would be useful in addressing the question of growth, survival and movement, although a successful method of tagging asteroids, especially juveniles, must first be designed.

All three species reproduce at the same time of year, March to early April, a pattern typical of most temperate water asteroids. Field observations indicate that adults of different species may release their gametes at the same time, suggesting that they are responding to the same spawning cues. The fact that S. stimpsoni degenerated oocytes rather than releasing them suggests that the three species are not responding to the same spawning cue, as there was little evidence retention of large oocytes, then degeneration in ovaries of the other two species. If they are responding to the same spawning que, then how do they remain reproductively isolated?

Barriers to hybridization do not appear to be prezygotic as there is an overlap in the time and place of spawning and sperm appear to be attracted to eggs of other species. The fact that cross fertilization is possible in all combinations indicates that the barrier, if present, must be postzygotic. Similarity in larval development may explain the ability of these species to hybridize. The low incidence of normal larval development in hybrid progeny suggests that these have reduced survivorship and that none may actually make it to reproductive maturity. As I was not able to raise them to maturity, due to time constraints, I cannot say whether they are capable of producing viable gametes or

offspring. In most organisms you expect that natural selection would rapidly promote the development of prezygotic mechanisms over postzygotic ones to prevent the waste of gametes. It is possible that a prezygotic barrier exists but has been overlooked, that one is presently evolving or that postzygotic mechanisms may be favored for some reason. In sea stars the loss of both eggs and sperm would be considerable due to external fertilization. If natural selection was acting to reduce the waste of gametes, you would expect that mechanisms of internal fertilization and copulatory organs would have evolved, all of which seem to be absent in sea stars. Further study is needed before any substantial conclusions can be made.

Larval development in sea stars of the species Crossaster papposus corresponds closely to that of Solaster spp., they occur in the same habitat and are spring spawners, suggesting that hybridization with Solaster may be possible. It would be interesting too, to follow the development of S. paxillatus, the fourth Solaster species in this area. I would expect the mode of development to be very similar, although it is separated in habitat somewhat from the other three species, as it is found in slightly deeper water. This species is rare in the Bamfield area and the chance of gametes encountering gametes from the same species would seem very low. It would also be interesting to compare the development of Lophaster furcilliger Fisher 1905, another uncommon species in the Family Solasteridae, found in the same habitat as S. paxillatus. Adults are considerably smaller than those of the four Solaster species, having a radius up to 82 mm and only 5 arms. Do all species in the Family Solasteridae in the Northeast Pacific Ocean exhibit the same mode of development and overlap with regards to season of reproduction? Is hybridization possible among all species in the Family Solasteridae?

Appendix 1 - Overview of Chronology of Development in Three Species of Solaster at 10-12 °C

Stage of Development	S. stimpsoni	S. dawsoni	S. endeca
4 - cell	3.5 h	6.5 h	5 (4-6) h
wrinkled blastula	26.5 (21-41) h	35 (28-48) h	32.5 (26-40) h
hatching	66.5 (65-68) h	49.5 (41-58) h	67.5 (54-84) h
brachiolarian	8 (7-9.5) d	10 (5.5-10) d	9 (6-10) d
attachment	21 (9-24) d	18 (8-26) d	20 (11-25) d
5 hydrocoel pouches	20 (17-24) d	13 d	12 d
8 sets tube feet	34 (27-40)d	22 (20-22) d	26 (20-31) d
10 sets tube feet	31 (27-45) d	39 (27-48) d	
loss of attachment	50 (45-54) d	37 (24-58) d	37 d
mouth opening	46 (40-54) d	50 (43-58) d	48 d
12 sets tube feet		53 (48-58) d	

* Developmental times (hrs, days) reported are the average (range) of those cultures which completed metamorphosis.